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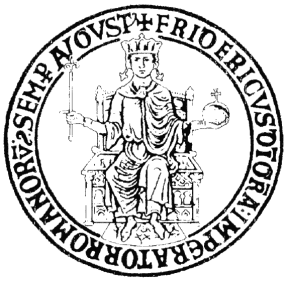

PhD Course in Biology

XXXVI Cycle

Identification of stem cell-specific markers for the molecular characterization of human Adult Gastric Stem Cells (hAGSCs)

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LIST OF ABBREVIATIONS

ESCs	**embryonic stem cells**
iPSCs	**induced pluripotent stem cells**
ASCs	**adult stem cells**
hESCs	**human embryonic stem cells**
HSCs	**hematopoietic stem cells**
GI	**gastrointestinal**
AGSCs	**adult gastric stem cells**
ECM	**extracellular matrix**
SPEM	**spasmolytic polypeptide expressing metaplasia**
hAGSCs	**human adult gastric stem cells**
hGOs	**human gastric organoids**
CF	**cystic fibrosis**
RNA-Seq	**single-cell RNA sequencing**
HPA	**Human Protein Atlas**
IF	**immunofluorescence**
IHC	**immunohistochemistry**
eGFP	**enhanced green fluorescent protein**
TTE	**TFF2 mRNA transcript-expressing**
GADC	**gastric adenocarcinoma**
IST	**intestinal stem cell**
AQP5	**aquaporin 5**
MUC6	**mucin 6**
A4GNT	**α1,4-N-acetylglucosaminyltransferase**
PGC	**progastricsin**
ELAPOR1	**endosome-lysosome associated apoptosis and autophagy regulator 1**
TMED6	**transmembrane p24 trafficking protein 6**
TMEM97	**transmembrane protein 97**
EDEM3	**ER degradation enhancing alpha-mannosidase-like protein 3**
TK1	**thymidine kinase 1**

LGR5	**Leucine-rich repeat-containing G-protein coupled receptor 5**
LRIG1	**Leucine-rich repeats and immunoglobulin-like domain 1** tumor
TNFRSF19	**necrosis factor receptor superfamily member 19**
SOX9	**SRY-box transcription factor 9**

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1. ABSTRACT

BACKGROUND: Human adult gastric stem cells (hAGSCs), due to their self-renewal capability and multipotency, play a significant role in stomach tissue regeneration and aging. However, hAGSC cellular homeostasis in gastric tissue physiopathology is poorly understood because the knowledge of their identity is still limited.

GOAL: The purpose of this work was to explore the biological dynamics of Neck, Chief, and Isthmus stem cells within a physiological context.

AIMS: To define the hAGSC cytotype-specific bio-markers, we performed bioinformatic data mining on open-access human gastric RNAseq single-cell databases and The Human Protein Atlas portal.

Furthermore, to standardize a reliable source of hAGSCs to characterize their molecular features, we generated organoids with bio-marker promoter sequences upstream of the eGFP target gene.

RESULTS:

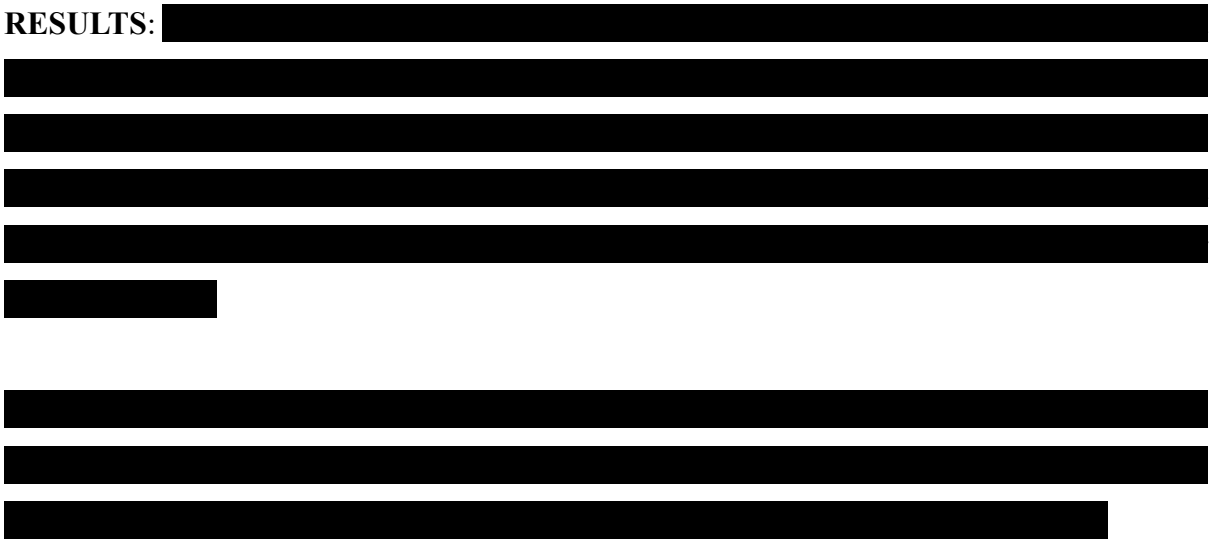

Figure 1 Graphical abstract

2. INTRODUCTION

Stem cells are non-specialized cells of the body. These cells can self-renewal and can differentiate in any cell of the organism, a capacity defined as developmental potency. Self-renewal allows them to contribute to a population of engaged cells while maintaining a stable population of stem cells. Specifically, when a stem cell divides, it results in two daughter cells, and there are three possible outcomes: two stem cells, one stem cell, and a more differentiated cell, or both differentiated cells. Instead, stem cell developmental potency refers to the ability of stem cells to differentiate into several cell types, which is reduced by passing from totipotent to unipotent stem cells. Totipotent stem cells represent the highest level of differentiation potential. They can give rise to all the cells of the organism. One example is the zygote, from which both embryonic and extra-embryonic tissues originate. Pluripotent cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), contribute to the three germ layers but not the extra-embryonic tissues (1). Multipotent cells can differentiate in different cells of specific cell lineages. The lowest differentiation capacity characterizes unipotent stem cells, which can form only one cell type. The stem cells in the adult organism are multipotent stem cells, defined as adult stem cells (ASCs) or "somatic stem cells."

Several scientific studies have expanded knowledge about stem cell physiology, but the road to a complete understanding of stem biology is still long. Discovering the mechanism behind self-renewal could help us understand how cell fate is regulated during normal embryonic development and post-natally or misregulated during aging or even in cancer development. In addition, stem cells find applications in the preclinical and clinical fields. They are used to understand the biology of disease and test therapy strategies and allow tissue engineering in regenerative medicine.

Human pluripotent stem cells, ESCs, and iPSCs provide opportunities for cell therapies against intractable diseases and injuries. In particular, the ESCs derive from the inner cell mass of mammalian blastocysts. Previous work with mouse embryos led to the development of a method in 1998 to derive stem cells from the inner cell mass of preimplantation human embryos and to grow human embryonic stem cells (hESCs) in the laboratory (2). These cells can grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers. However, the use of ESCs for research purposes is limited by various factors, such as the ethical and social problems related to the use of human embryos and immune rejection

after transplantation. To overcome these issues, Takahashi and Yamanaka induced pluripotency in somatic cells through specific transcriptional factors present in ESCs, the so-called Yamanaka transcription factors OCT4, SOX2, KLF4, and MYC (OSKM factors), and they designated this new type of pluripotent stem cell as iPSCs (3). The iPSCs are pluripotent stem cells generated by direct reprogramming of terminally differentiated somatic cells. The reprogramming determines a reversal of the cell gene expression profile. Thus, differentiation-related gene activity is turned off as undifferentiation-related genes are activated. However, even the iPSCs, like the ESCs, have features that limit their use, including their intrinsic properties of immunogenicity, heterogeneity, and, especially, tumorigenicity (4).

Finally, ASCs also have many potential in the medical field. Until now, the most well-characterized example of ASCs is the hematopoietic stem cells (HSCs) (5). Hematopoietic stem cell transplantation is still the most popular application of stem cell therapy. HSC transplantation solves problems that are caused by inappropriate functioning of the hematopoietic system, which includes diseases such as leukemia and anemia (6).
The promise of stem cells in future therapies is exciting, but significant technical hurdles will likely only be overcome through years of intensive research.

2.1 Adult stem cells

Adult stem cells are a small tissue-resident population of multipotent stem cells (7). Some (such as the liver stem cells) show very low proliferative activity but undergo over-activation of proliferation in case of damage. Others (such as the gastrointestinal - GI -epithelium stem cells) are constantly self-renewing with tissue replacement rates even in the order of days (8). There is clear evidence of a resident stem cell population for some tissues such as the intestine (9), liver (10), and mammary gland (11). In these organs, they can differentiate to produce progenitor, precursor, and mature cells, but this is limited to the cells contained in the tissue of origin. Throughout the life of these organs, populations of adult stem cells serve as an internal repair system that generates replacements for cells lost through normal wear and tear, injury, or disease (12).

2.2 Adult gastric stem cells

Replenishing new epithelial cells in the gastric luminal environment is essential to maintain organ structure and function during routine turnover and injury repair. This is necessary due to damage caused by ingested food, gastric acid, or digestive enzymes. The GI has the highest turnover rate among adult mammals' endodermal tissues (13).

Following the discovery of intestinal stem cells, recent studies using lineage tracing models have identified diverse populations of stem cells and even differentiated cells that can regain stem cell capacity. However, the molecular identity of gastric stem cells still needs to be determined (14). Adult gastric stem cells (AGSCs) represent the adult stem cell population that resides in the stomach's mucosa, allowing the regeneration and repair of this tissue. The human stomach is divided into four anatomical districts: *cardia*, *corpus*, *antrum,* and *pylorus* (**Fig.2**).

Figure 2 A schematic representation of the human stomach.
The stomach has four main anatomical districts: the *cardia*, *fundus*, *corpus*, *antrum,* and *pylorus*. The *cardia* surrounds the superior opening of the stomach, and it is the point where the *esophagus* connects to the stomach. *Fundus* is the dome-shaped often gas filled portion superior to and left of the *cardia*. Below the *fundus* is the *corpus*, the large central portion of the stomach. The distal area of the stomach consists of the *antrum,* pyloric canal, and pyloric sphincter, which connects to the duodenum.

The gastric mucosa, in the four anatomical districts, is characterized by a single columnar layer with numerous invaginations in the *lamina propria*, which are called gastric units (15). The gastric units consist of four parts: from the lumen, they are named the pit, isthmus, neck, and base region. While pit and isthmus are relatively similar in all glandular gastric units, the cellular composition of the neck and base varies between the stomach areas, reflecting the specific functions of these regions (16). Two staminal niches that differ in their localization

coexist in the gastric unit, both in the *corpus* and *antrum* (**Fig.3**) (14): one in the *isthmus* region and the other at the base of the gland, although the precise features of the two niches are currently under debate.

Figure 3 A schematic representation of the gastric units in different anatomical regions (*antrum vs. corpus*) of the human gastric epithelium.
Adult *corpus* units primarily contain Parietal cells (blue) and Chief cells (red), but also Pit cells (pink), Isthmal and basal stem cells (white), Mucous cells (green) and Endocrine cells (yellow). Adult antral units primarily contain Mucous cells (green), but also pit cells (pink), proliferative isthmal and basal stem cells (white) and Endocrine cells (yellow). To a lesser extent, antral units present Parietal cells (blue) and Chief cells (red).

In both these two niches, there are two stem populations with different functions (**Fig.4**): one actively proliferating, located mainly at the level of the isthmus, whose cells migrated bidirectionally, constituting all the cells of the pit and base region, and allowing the physiological replacement of the gastric mucosa; and a quiescent population (out of cell cycle and in a lower metabolic state) which can be activated either by a stochastic mechanism or by feedback upon loss of active stem cells or in response to injury and pathological challenge (17).

Figure 4 Stem cell population in the human gastric units.

Quiescent and active stem cells coexist within the gastric tissue. Quiescent stem cells are cells out of cell cycle and in a lower metabolic state. Active stem cells are cells in cell cycle. active stem cells are the primed subpopulation that account for most of the replenishment of corresponding tissues, whereas quiescent stem cells function as a reserved sub-population.

In adults, all gastric units are considered to be monoclonal, meaning that all cellular progeny is derived from a single stem cell. Contrary to the belief that stem cells divide asymmetrically to preserve one stem cell and one amplifying daughter cell destined to differentiate, lineage tracing models showed that gastric stem cells divide symmetrically to produce two phenotypically equal daughter cells. The neutral competition for niche space permits only daughter cells that stay within the niche to remain as stem cells (14).

Out of the stem cell niche, six differentiated gastric cell types are present: pepsinogen-secreting zymogenic cells or Chief cells that secrete the digestive enzymes pepsinogen; acid-producing oxyntic cells called Parietal cells; surface mucous cells named Pit cells; Mucous Neck cells, that also produce mucus in the inner part of the unit; Endocrine cells (which include the gastrin-secreting G cells, somatostatin-secreting D cells, serotonin-secreting enterochromaffin EC cells, histamine-producing EC-like cells and ghrelin-secreting X/A cells), that are dispersed throughout all glands but account for < 2% of the epithelium; and, finally, the rare Tuft cells that express chemosensory markers and characteristic apical microtubules, whose function, however, has not yet been well defined (16).

2.3 The gastric stem cell niche

The stem cell niche is the *in vivo* microenvironment where stem cells reside and receive stimuli that determine their fate. Therefore, the niche should not be considered simply a physical location for stem cells but rather a place where extrinsic signals interact and integrate to

influence stem cell behavior. These stimuli include cell-to-cell and cell-matrix interactions and signals that activate or repress genes and transcription programs. As a direct consequence of this interaction, stem cells are maintained in a dormant state, induced to self-renewal or commit to a more differentiated state (18). The general niche model involves the association between resident stem cells and heterologous cell types (niche cells). Preserved components of the niche are the supporting stromal cells that secrete fundamental molecules; the extracellular matrix (ECM), which constitutes a mechanical framework unit to transmit stem cell signaling; blood vessels that carry nutritional support and systemic signals to the appropriate place; and the neural inputs, that promote the mobilization of stem cells from their niches and integrate signals from different organ systems (19). In the stomach, Sonic Hedgehog (SHH) and bone morphogenetic protein 4 (BMP4) shape the gastric stem cell niche (**Fig.5**).

Figure 5 Human gastric stem cell niche.

Distribution of niche factors in the corpus gland. Circles indicate the area in which niche factors are secreted. The SHH-secreting parietal cells lead to the formation of a gradient of SHH. SHH then regulates mesenchymal BMP expression. There is only indirect data available for the proposed WNT gradients shown in this illustration, and the source of WNT is unclear.

SHH is secreted only by Parietal cells in the neck region of the gastric unit, and its receptor Patched 1 (PTC1) is expressed by mesenchymal cells and gastric epithelial cells in the same area. According to this expression pattern, the hedgehog regulated the expression of the H^+/K+-ATPase in parietal cells and the secretion of BMP4 by mesenchymal cells. The SHH-BMP4 axis inhibits cellular proliferation and promotes cellular differentiation. On the other hand, SHH signaling forms a harmful mold for the stem cell zones, which can be established only

outside the parietal cell/SHH/BMP4 zones, in the isthmus, and at the base of the gland (20). In gastric development, Hh signaling also counteracts the activity of Notch, which sustains gastric stem cell maintenance in the isthmus and the base region of antral units (21) (22). In addition to BMP and NOTCH, the third pathway regulated by SHH is represented by the fibroblast growth factor 10 (FGF10). In the development of the adult stomach in mice, FGF10 is expressed in the mesenchyme, and the knockout of FGF10 or its FGFR2 receptor leads to abnormal development of the gastric unit (23). Other niche factors that promote stem cell populations include WNT and its target genes (such us LGR5 and TROY), whose expression is reduced during stomach development. However, it is expressed within the gastric units of the *corpus* and the *antrum* (24). Epidermal growth factor (EGF), including the transforming growth factor alpha (TGF-α), plays a significant role in gastric cell proliferation (25) (26). Finally, Gastrin (Gast), whose receptor (CCK2 receptor) is present on Parietal cells and enterochromaffin-like (ECL) cells in the *corpus*, but also in both neck progenitors and stem cells in the gland base (27).

2.4 Gastric stem cell markers

A set of markers labels populations of stem cells at the base and neck region of the glands. Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) gene is a WNT target gene. In mammals, physiological Wnt signaling is intimately involved with the biology of adult stem cells and self-renewing tissues. Lgr5 resides in Wnt receptor complexes and mediates signaling of the Wnt-agonistic R-spondins. When bound to Lgr5, R-spondin captures and inactivates RNF43/ZNRF3, downregulating Wnt receptors in a negative feedback loop (28) (29). The gene Lgr5 marks continuously cycling stem cells at the base of intestinal and colon crypts (9) and other tissues, including hair follicles (30), pancreas (31), prostate (32), and liver (33) (34). The stomach shares a common endodermal origin with the intestine and a constantly renewing epithelium like the intestine (under physiological conditions, gastric epithelial cells undergo continuous dynamic renewal within as little as three days). Also, in this organ, Lgr5 marks active-cycling adult stem cells at the base of pyloric glands. *In vivo* mouse lineage-tracing models demonstrate that the $Lgr5^{+}$ cells at the gland base are active, multipotent stem cells contributing to the long-term renewal of the gastric epithelium in the pyloric region. The Lgr5 progeny generated at the gland base rapidly migrate to the isthmus, where they undergo transient amplification before committing to the various functional lineages during bidirectional migration toward the pit or the gland base (24). Lgr5 marks WNT-dependent stem

cells that drive constitutive physiological tissue self-renewal but also define a class of stem cells or progenitors that is called into action upon tissue damage. In particular, Lgr5 marks a subpopulation of Chief cells in the *corpus*-type gastric units, representing a quiescent stem cell population capable of reactivating in the event of damage (35).

An additional stem cell marker that was identified because it is co-expressed with Lgr5 in the intestine is the gene tumor necrosis factor receptor superfamily member 19 (Tnfrsf19), which is highly expressed at the base of *corpus* glands with Lgr5. Troy is expressed by a small subset of Chief and Parietal cells located at the gland base. Troy$^+$ Lgr5$^+$ Chief cells at gland bottoms can serve as quiescent stem-like cells in the epithelium of the gastric *corpus*, as they are fully differentiated cells capable of acting as a multi-potent stem cell, re-entering the cell cycle. In a remarkable example of cellular plasticity, these Chief cells spontaneously de-differentiate to act as multipotent epithelial stem cells *in vivo*, particularly upon damage. Troy$^+$ Chief cells are activated following damage at the level of actively proliferating stem cells in the isthmus (as expected by a reserve stem population) and upon the specific loss of Parietal cells that leads to the generation of a metaplastic cell lineage called SPEM (spasmolytic polypeptide expressing metaplasia) (36). Troy$^+$ Chief cells are unique among Chief cells in expressing many Wnt target genes and producing all epithelial lineages in the *corpus in vivo* (35). Another population of reserve stem cells was marked with the gene villin (Vil1) at the base and neck of antral glands. Vil1-positive cells are rare and cycle only slowly, which most likely does not contribute to homeostasis but may act as a reservoir of stem cells for repair after injury. This group of stem cells is mainly located in the third part of the glands, with lineage tracing showing that they can differentiate into all types of cells in the antral glands (37). Just above the Lgr5-expressing zone, the sex-determining region Y (SRY)-box2 (SOX2) marks rare cycling cells at the base of the pyloric and *corpus* gland (38). The cholecystokinin B receptor (CCKBR) marks a rapid cycling population of base stem cells that contribute to maintaining epithelial renewal in pyloric regions under normal homeostatic conditions (27). Axin2$^+$ cells are at the base and lower *isthmus* of the antral gland. These cells overlap with Lgr5$^+$ stem cells; Axin2$^+$ Lgr5$^-$ cells can give rise to Lgr5$^+$ cells; the high proliferation rate of Axin2$^+$ cells depends on Wnt agonist R spondin (39).

A new marker of pyloric stem cells, aquaporin 5 (AQP5), has been identified. AQP5 regulates water transport in healthy tissues and is also implicated in tumors as a driver of proliferation and invasiveness *in vitro* (40). AQP5 marks mouse and human LGR5$^+$ adult *antrum* stem cells. Lineage tracing detailed the homeostatic behavior of AQP5$^+$ cells that generate the major

pyloric lineages, confirming multipotency in the AQP5$^+$ population. Tracing is absent from the *corpus* (41). As in the base/neck region and also in the isthmus region, there are several markers of stem populations. The trefoil factor family 2 (TFF2) transcript may be the candidate for the adult stem cell markers in the isthmus mucosa. Lineage tracing demonstrated that TFF2 mRNA-expressing cells in the isthmus region are the progenitors for Mucus neck, Parietal, and Chief cells but not for Pit or ECL cell lineages in the *corpus*. At the same time, TFF2 protein is mainly expressed and secreted by Mucus neck cells and deep antral gland mucus cells under normal conditions (42) (43). In the isthmus region, the muscle intestine and stomach expression 1 (MIST1) marks a stem cell population of *corpus* gastric units, which plays a critical role in the differentiation of *corpus* glands. Most Mist1$^+$ stem cells in *isthmus* are quiescent under normal conditions, giving rise to the entire gland in response to gastric injury. Moreover, MIST1 marks a subpopulation of Chief cells and is necessary for their development (44). Leucine-rich repeats and immunoglobulin-like domain 1 (LRIG1)-expressing isthmal cells (in the *corpus* and antral gland) can contribute to the regeneration of Parietal cells following acute gastric injury (45). Runx1 enhancer element (eR1) - cells in the isthmus of the *corpus* and the base of the pyloric gland are reported to be involved in tissue regeneration and continuously give rise to mature cells that maintain gastric units (46). Bmi1-expressing cells in the isthmus of the gastric *antrum* and *corpus* also provide progeny bipolarly and are required for the homeostasis/regeneration of gastric epithelium (47). More recently, the left-right determination factor 1 (LEFTY1) was identified as a marker of a Neck cell subpopulation showing stem cell characteristics. LEFTY1$^+$ cells were present at low frequencies in gastric pyloric and fundic glands and seemed to be located around and in the isthmus regions (48).

The stem cell populations in the human gastric mucosa still need to be fully understood.

2.5 Human ASC-derived organoids in the study of adult gastric stem cell biology

Recent advances in 3D culture technology allow adult stem cells to exhibit their remarkable self-organizing properties, and the resulting organoids reflect critical structural and functional properties of organs. An organoid is now defined as a 3D structure grown from stem cells and consisting of organ-specific cell types that self-organize through cell sorting and spatially restricted lineage commitment (49). Organoids can be initiated from single stem cells, cultured long-term, and cryopreserve, and are amenable to all cell-biological and molecular analyses developed for 2D models, representing the right compromise between cell lines and *in vivo* studies.

Organoid cultures have been generated from several adult epithelial tissues, such as intestine and stomach (24) (50) (35), gallbladder (51), liver (33) (34), and pancreas (52). Organoid cultures were also generated from other non-gastrointestinal epithelial tissues such as the esophagus (53), fallopian tube (54), mammary gland (55), lung (56), prostate (57), salivary gland (58), and taste buds (59). Organoids can also be cultured from ectoderm organs, for example, brain (60) and retina (61) organoids.

Organoid cultures can be generated from the two main types of stem cells: pluripotent embryonic stem cells or induced pluripotent stem cells (brain and retina organoids), and multipotent adult stem cells (for example, esophagus, fallopian tube, small and large intestines, liver, pancreas, prostate, and stomach). Both approaches (from pluripotent and multipotent adult stem cells) exploit normal stem cells' seemingly infinite expansion potential in culture. In particular, gastric ASC-based organoids provide fundamental insights into the processes that allow this stem cell population to maintain and repair gastric epithelium. ASCs can be coerced to form organoids by creating conditions that mimic the stem cell niche environment during physiological tissue self-renewal or damage repair. The Wnt pathway has emerged as the primary driver of endodermal ASCs (62), and LGR5 (a Wnt target gene) marks active ASCs in many epithelia, including gastric epithelium: proliferating $Lgr5^{+}$ stem cells, located at the base of pyloric glands of the adult mouse stomach, efficiently generate long-term, continuously expanding organoids closely resembling mature pyloric epithelium (24); single $Lgr5^{+}$ $Troy^{+}$ Chief cells can be cultured to generate long-lived mouse gastric organoids, containing the various cell types of *corpus* glands (35); $LGR5^{+}$ $AQP5^{+}$ pyloric cells routinely established organoids that were passaged for more than three months (41). Unsurprisingly, Wnt activators (such us Wnt3A and R-spondins) are critical components of most ASC culture growth factor cocktails, including human gastric ASC-derived organoids. As previously mentioned, the niche factors WNT, EGF, FGF-10, GAST, and inhibition of BMP are essential for the *in vitro* reconstitution of the gastric stem cell niche, which has led to the development of the long-term adult gastric stem cell organoid culture (50).

Ex vivo expanded gastric adult stem cell-derived organoids retain their organ identity and genome stability and can be differentiated to resemble all cellular lineages present in the pertinent organ. Therefore, gastric ASC-derived organoids could serve as an unlimited source for studying the self-renewal and regenerative potential of human gastric adult stem cells in both physiological and pathological conditions (50). Because of the close resemblance to

human organs in health and disease, organoids hold great appeal for translational research and invite an almost immediate application into the clinic (**Fig.6**). Organoids open up new avenues for regenerative medicine in combination with editing technology, for gene therapy. An application of ASC-derived organoids in regenerative medicine combined with gene therapy was the gene correction in cystic fibrosis (CF) patients with a single-gene hereditary defect: CRISPR/Cas9 gene editing was utilized to correct a CFTR mutation in small intestinal and rectal organoids from two patients (63). Still, mouse colon organoids expanded *in vitro* from a single stem cell can, upon transplantation into multiple damaged mouse colons, engraft and develop into functional crypt units, fully recovering epithelial barrier function (64). Similar results were obtained using human adult stem cell-derived liver organoids in immune-deficient mice whose liver was chemically damaged, yielding functional hepatocyte grafts (34).

Moreover, ASC-based organoid technology plays a role in the personalized medicine field. Organoids allow rapid *ex vivo* testing of drug responses on the affected tissue of individual patients. For example, ongoing trials will reveal the validity and applicability of tumor organoids in assessing drug response at the level of the individual patient (65).

Figure 6 Organoids applications.

3. AIM OF THESIS

4. RESULTS

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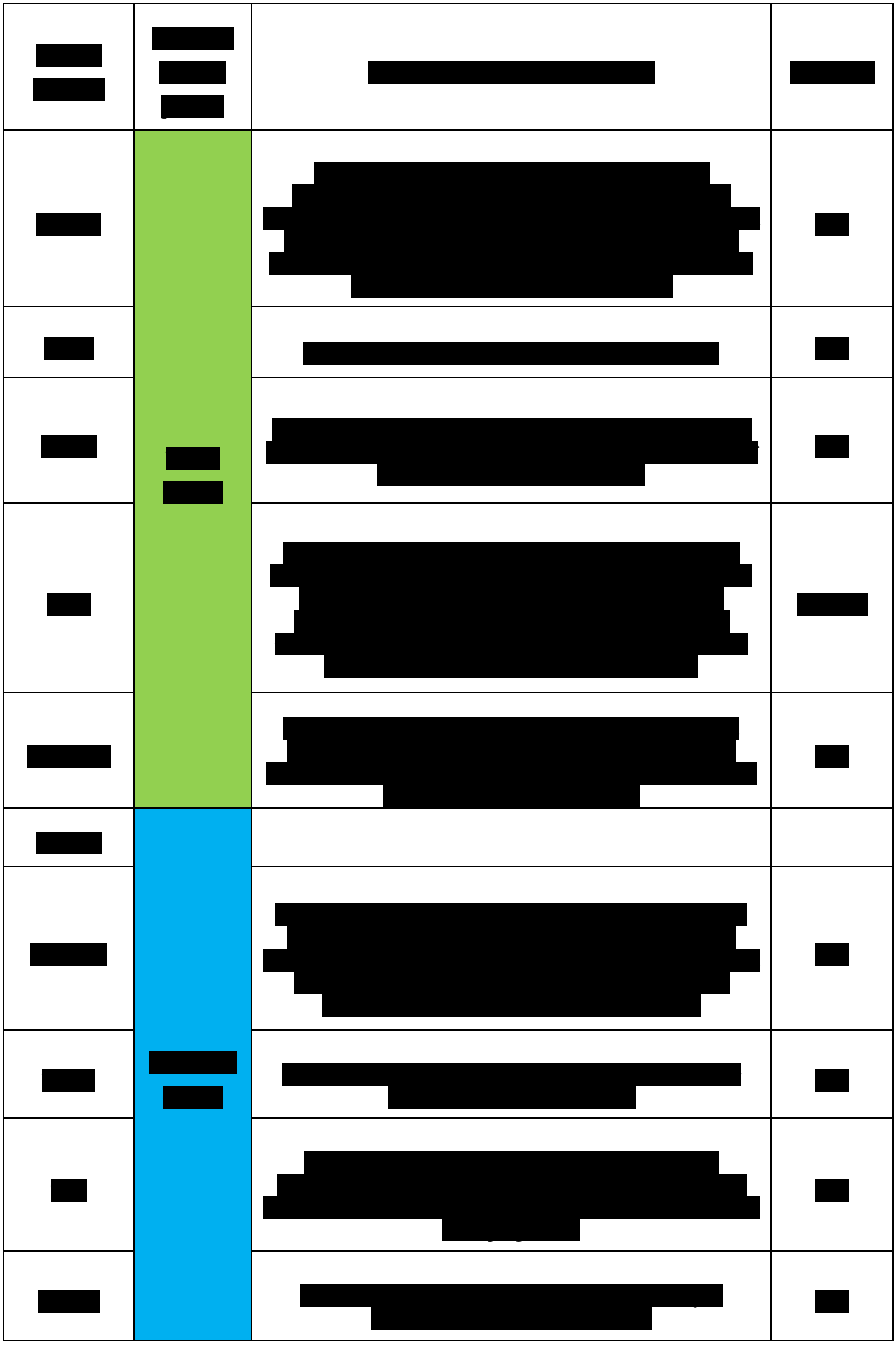

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The candidate neck cell markers AQP5 and MUC6 coexpression with the well-established stem marker SOX9 was examined to further characterize these cells in the stomach. The positive AQP5 and MUC6 populations partially expressed nuclear SOX9, as **Figures 10 A** and **B** show.

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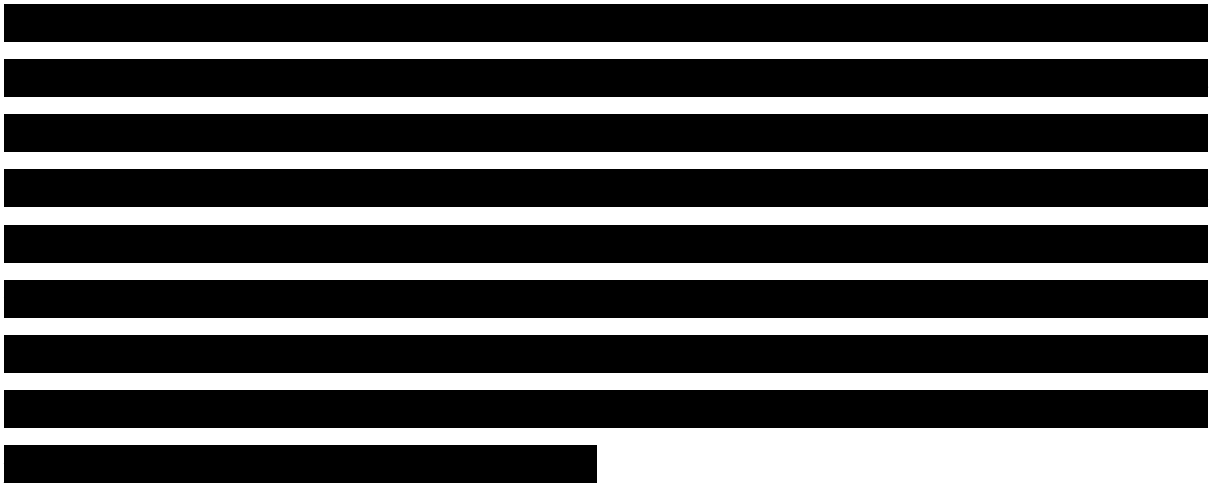

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7. MATERIAL AND METHODS

7.1 Human Tissue Material

Gastric tissues were obtained from putative healthy patients. The study was approved by the ethics committees of Hospital IRCCS - CROB (Rionero in Vulture (PZ), Italy) and Sant'Ottone Frangipane (Ariano Irpino (AV), Italy). Written informed consent was obtained from all patients before specimen collection.

7.2 Gastric glands processing

The gastric biopsies were collected in chelating buffer (Na_2HPO_4 5.6 mmol/L, KH_2PO_4 8.0 mmol/L, NaCl 96.2 mmol/L, KCl 1.6 mmol/L, Sucrose 43.4 mmol/L, Dsorbitol 54.9 mmol/L, and DL-dithiothreitol 0.5 mmol/L in filtered MilliQ water, pH 7). After the gastric biopsies, they were kept on ice until the dissociation procedure. The gastric specimen was aspirated with a 1000µl tip during the dissociation procedure, and the biopsy was cut into small pieces. The tissue was dissociated in a GentleMACS type C tube with 2.5mL of dissociation medium (containing collagenase, hyaluronidase, P/S 100U, and Gentamicin 50µg/mL), in gentleMACS Dissociator with "37°C_tumor one program".

The biopsy was placed at 37°C for 1:30h in agitation (800 rpm). The single cells, aggregates, and undigested pieces were seeded in Matrigel drops into a pre-warmed 24-well plate and overlayed by a complete medium (**Tab. A**) plus RHOKi.

7.3 Organoid Culture

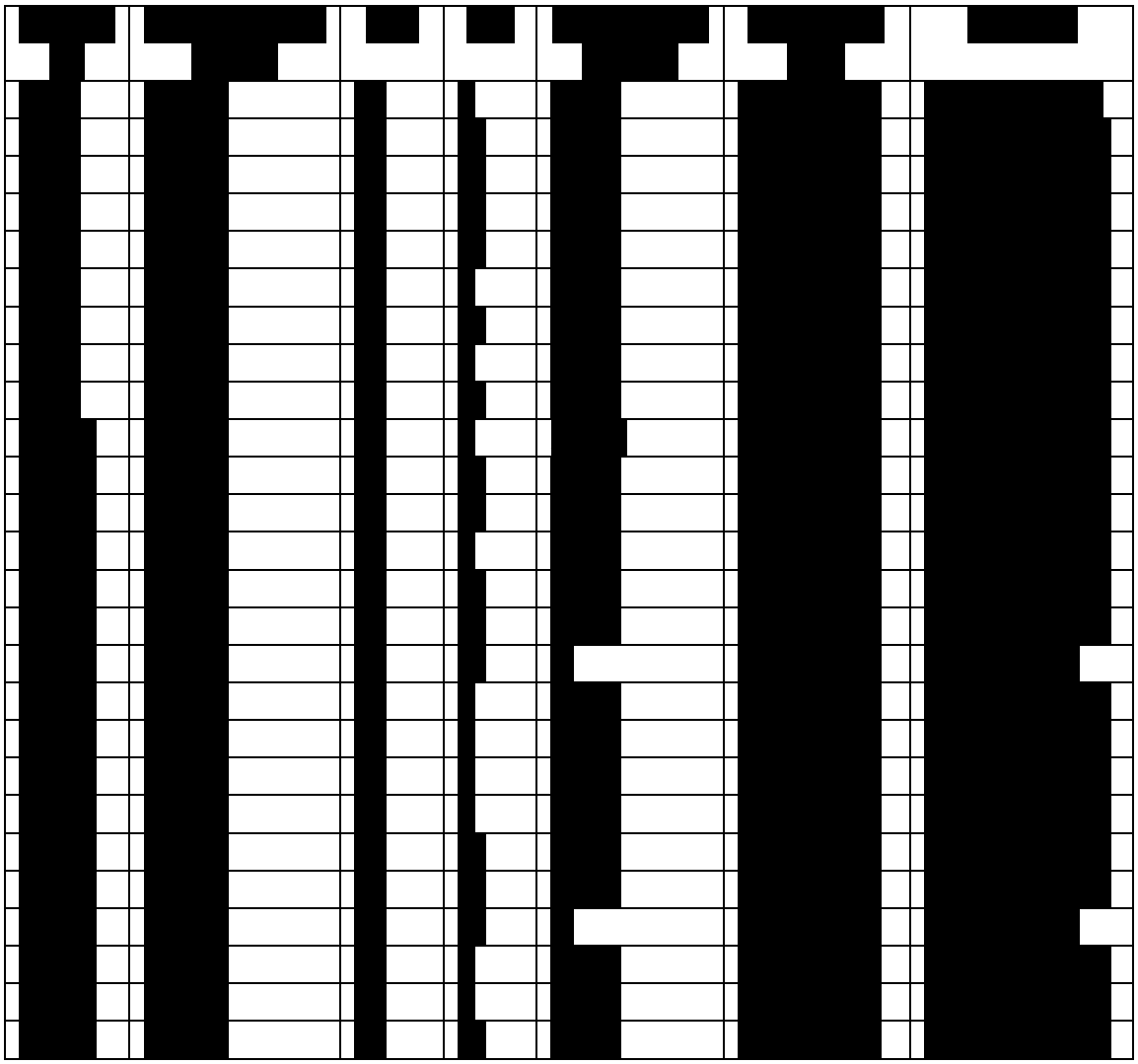
After dissociation of human gastric biopsy single cells, aggregates and undigested pieces were seeded in a pre-warmed 24wells plate with 30µL Matrigel drop, which was left to solidify at 37°C for 10 min. The drop was overlying with 500µL of complete medium (**Tab. A**). After seeding, the plate was transferred to the incubator at 37°C and 5% CO_2. The medium was refreshed every 2-3 days (3 times per week).

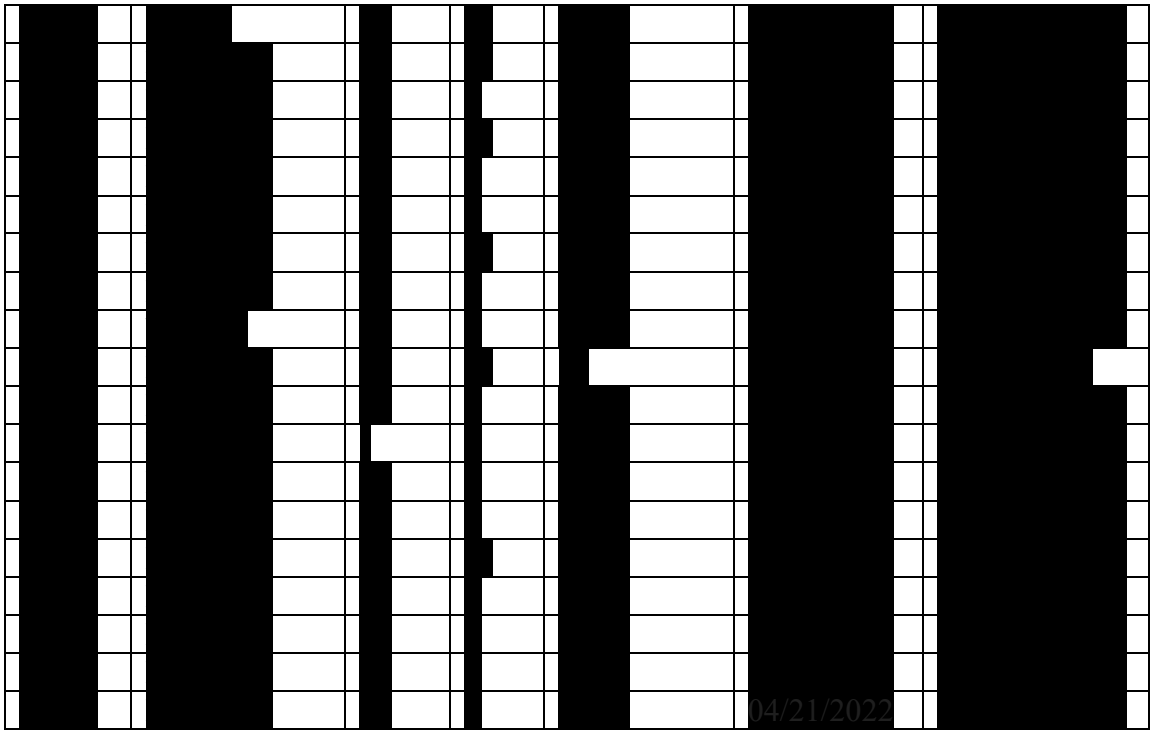
Component	Supplier	Catalogue Number	C_f
Advanced DMEM/F12	Gibco	12634-028	
Wrn medium			50%
B27 supplement	Gibco	17504-044	1X
P/S	Gibco	15140-122	100U
Y-27632	Tocris	1254	10µM
GlutaMAX	Gibco	35050-079	1X
Nicotinamide	Sigma	N0636	10mM
N-acetylcysteine	Sigma	A9165-5G	1mM
H. recomb. FGF10	Peprotech	100-26	200ng/ml

M. recomb. EGF	Gibco	PMG8043	50ng/ml
[Leu15]-Gastrin	Sigma	G9145	1nM
A-83-01	Tocris	2939	2µM
HEPES	Gibco	15630-056	10mM
Matrigel	Corning	356255	
TrypLE express	Gibco	12604-013	
Recovery Cell Culture Freezing Medium	Gibco	12648-010	
L-WRN	ATCC	CRL-3276	
Collagenase IV	Gibco	17104019	1.5mg/mL
Hyaluronidase V	Sigma	H6254	20µg/mL

Tab.A: Human gastric organoid medium reagents.

Forty-five lines of human gastric organoids (antrum and corpus) were generated. **Table B** provides more details about the patients from whom the lines were generated.





Tab.B: Human gastric organoid lines.

7.4 Passage, freezing, and thawing of organoids

Human gastric cultures were split 1:2 about every 14 days. Organoids were mechanically or enzymatically dissociated for passage and then transferred to fresh Matrigel. The basement matrix was broken using a 1 mL micropipette. Organoids were transferred to a 15 mL tube with 5 mL of basal medium and were centrifuged for 5 min at 1500 rpm at 4°C. Organoids were cellularized using TrypLE express; after 10/15 min, trypsin was inactivated by adding 1 mL of basal medium. The dissociated cells were centrifuged for 5 min 1500 rpm at 4°C. The pellet was suspended in an appropriate amount of Matrigel. The drop was placed in a 24-well. The basement matrix was left to solidify at 37°C for 10 min. The drop was overlaying with 500µL of complete medium. All previous operations must be conducted on ice to prevent Matrigel from solidifying.

For freezing, organoids were dissociated as previously described for splitting. The dissociated cells were suspended in 500µL of Recovery Cell Culture Freezing Medium and were stored at -80°C after 24h in liquid nitrogen.

7.5 Plasmid construction

The plasmid pLenti CMV GFP Puro (658-5) #17448 (containing the constitutive lentiviral CMV promoter and its enhancer) was obtained from Addgene (Cambridge, MA, USA). The PGC (1467 bp), A4GNT promoter (4966 bp), and MUC6 promoter (2643 bp) sequence were amplified from AGS cell line gDNA with Wonder Taq Thermostable DNA polymerase (Euroclone), using the primers listed in **Table C**. The cytotype-specific plasmids (pLENTI_PGCpromoter_eGFP; pLENTI_A4GNTpromoter_eGFP and pLENTI_MUC6promoter_eGFP) were generated with Takara Bio's In-Fusion HD Cloning, excising the CMV promoter. The plasmid containing the sequence for the AQP5 gene promoter (2851 bp) was synthesized by GenScript Biotech (Leiden, Netherlands). The original plasmid pLenti CMV GFP Puro (658-5) #17448 was sent to the company as a spot on 3mm paper (with centration of about 8ug), and the promoter sequence synthesized and subcloned between the ClaI site and the EcoRV site (5'-3' direction) after removing the CMV.

Gene	Sequence
hPGCp	Fw 5'- GCCGGCCGGCCGTTTAAACAGCAGTGGCTAGAGTGGAAAATCC -3'
	Rv 5'- CCGGTGGATCCGTTTAAACGATGCTGGTCCCCAACTGG-3'
hA4GNTp	Fw 5'- GCCGGCCGGCCGTTTAAACTCTGGGGCACAACTGGTTTATAGG -3'
	Rv 5'- CCGGTGGATCCGTTTAAACGTCCTCTTCTCTGTGCCAGCC-3'
hMUC6p	Fw 5'- GCCGGCCGGCCGTTTAAACGGATGCACAGCTTTCACCAG -3'
	Rv 5'- CCGGTGGATCCGTTTAAACGGTGCACAGTGGAGAGGAG-3'

Tab.C: Amplification promoter primers.

7.6 Organoid Infection

Organoids were trypsinized and transferred into a well on a 48-well plate. 150µl of transduction medium and 150 µl of standard medium plus polybrene 1:1000 were added to one well of organoids in a 15mL falcon tube. To enhance transduction efficacy, spinoculation was performed by putting the 15mL falcon tube containing organoids in a pre-warmed centrifuge at 32 °C and rotating at 600 x g for 1 hr. The organoid-virus mixture was placed in a culture incubator for 3 hr at 37 °C to allow transduction. The organoid-virus mixture was placed in 30µl of ice-cold matrigel with a complete medium without polybrene. After 24h, virus medium

cleanup was performed. After 72h, the medium was refreshed and supplemented with a selection antibiotic (1µg/mL puromycin). The selection was lowered one week after the infection (500ng/mL puromycin).

7.7 RNA extraction, RT-PCR, and Real-Time PCR

RNA was extracted from organoids, cells, gastric and colon biopsy using RNeasy Mini kit (Qiagen) according to the manufacturer's recommendations. cDNA was conducted using iScript cDNA Synthesis Kit (BioRad) according to the manufacturer's recommendations. PCRs were performed using Wonder Taq Thermostable DNA polymerase (Euroclone). Real-time PCR was performed with the iTaqTM Universal SYBR® Green Supermix (Biorad) using an AriaMx Real-time PCR System (Agilent, Santa Clara, California, USA), under the following conditions: 95°C, 10'; (95°C, 15''; 60°C 1') x 40 cycles; 95°C, 15"; 60°C, 1'; 95°C, 15". Real-time data were analyzed using AriaMx Real-Time PCR Software (Agilent). Reactions were carried out in triplicate, and gene-expression levels were calculated relatively to β-Actin mRNA levels as endogenous control. Real-time PCR amplification values were reported as $2^{-\Delta Ct}$, where ΔCt is Ct_{gene} under investigation$-Ct_{endogenous\ control}$.

Primers used for RT-PCR and Real-time PCR are in the **Table D.**

Gene	Fw	Rv
hLGR5	5'-TATGCCTTTGGAAACCTCTC-3'	5'-CACCATTCAGAGTCAGTGTT-3'
hLRIG1	5'-AACCGTCGTGCCACCAGATGTTCC-3'	5'-GCTGCACTGGTGATGTGGAGAATGC-3'
hPGC	5'-AGAGCCAGGCCTGCACCAGT-3'	5'-GCCCCTGTGGCCTGCAGAAG-3'
hMUC6	5'-GCCCCGGTATCTTCTCTCGG-3'	5'-ACACCTGCAGGGTGAGTACG-3'
hMUC5AC	5'-CTTCTCAACGTTTGACGGGAAGC-3'	5'-CTTGATCACCACCACCGTCTG-3'
hTFF1	5'-CCATGGAGAACAAGGTGAT-3'	5'-CACCAGGAAAACCACAATTC-3'
hTFF2	5'-GACAATGGATGCTGTTTCG-3'	5'-GTAATGGCAGTCTTCCACAGA-3'
hTROY	5'-CCTCCTCCTCCTTACGAACC -3'	5'- TGACACAGAGGATGAGCAGG-3'
hAQP5	5'-TACGGTGTGGCACCGCTCAATG-3'	5'-TCAGCGGGTGGTCAGCTCC-3'
hA4GNT	5'-GTGGCCTGTGGTGTTCTTT-3'	5'-GTGGAGCCAGTTTCTCTCTG-3'

hCDX1	5'-GTGGCAGCGGTAAGACTC-3'	5'-GTTCACTTTGCGCTCCTTTGC-3'
hACTIN	5’-GAGCACAGAGCCTCGCCTTT-3’	5’-TCATCATCCATGGTGAGCTGG-3’
hSST	5'-CTAGAGTTTGACCAGCCAC-3'	5'-GACAGATCTTCAGGTTCCAG-3'
hNANOG	5’-AGCTACAAACAGGTGAAGAC-3’	5’-TAGGAAGAGTAAAGGCTGGG-3’
hOCT4	5’-TCTTCAGGAGATATGCAAAGC-3	5’-ATCCTCTCGTTGTGCATAGT-3’
hSOX2	5’-CAAGGAGAGGCTTCTTGCTGA-3	5’-CACAGAGATGGTTCGCCAGT-3
hSOX9	5'-ACGGCTCCAGCAAGAACAAGC-3'	5'-TCTTCACCGACTTCCTCCGC-3'
hZSCAN4	5’-ATCCACCTGCCTTAGTCCAC-3	5’-TCGAAGAACTGTTCCAGCCA-3’
hCD44	5'-AGATGGAGAAAGCTCTGAGC-3'	5'-GGTAATTGGTCCATCAAAGGC-3'
hCD133	5'-CTTAATATCTTTCTGTTGGG-3'	5'-TTGCTTCTAGATCATATGC-3'
hCBLIF	5'-CCTTGGCTCTGACCTGTATGT-3'	5'-CCGTAGTCTTCTTGCAGTTCC-3'
hGAPDH	5'-GGTATCGTGGAAGGACTCATGAC-3'	5'-ATGCCAGTGAGCTTCCCGTTCAG-3'

Tab.D: Gastric marker expression primers.

7.8 Immunohistochemistry and immunofluorescence

Immunofluorescence Immunofluorescence (IF) was performed according to standard protocols. In summary, tissues were fixed in 4% paraformaldehyde in PBS (w/v) overnight at 4 °C and processed into paraffin blocks. Five-micrometer sections from the paraffin blocks were deparaffinated and rehydrated, followed by antigen retrieval in standard 0.1M citric acid pH6 buffer. The primary antibodies used were rabbit anti-aquaporin 5 **(Tab. E)**.

Anti-body	**Supplier**	**Catalogue Number**	**C_f**
Anti-AQP5	Sigma, Santa Cruz	HPA065008, Sc-514022	1:100
Anti-PGC	ABCAM	AB180709	1:100
Anti TROY	Santa Cruz	Sc-398526	1:50
Anti-A4GNT	Sigma	HPA008017	1:100
Anti-MUC6	Santa Cruz	Sc-33668	1:100
Anti-EDEM3	Sigma	HPA025754	1:50
Anti-TK1	Santa Cruz	Sc-377211	1:50
Anti-TMED6	Sigma	HPA012532	1:50
Anti-LIPF	Sigma	HPA045930	1:500
Anti-ELAPOR1	Sigma	HPA029869	1:250
Anti-TMEM97	Sigma	HPA076689	

Anti-TFF2	Sigma	HPA036705	1:1000
Anti-CDH1	Cell Signaling	24E10, 4A2	1:200
Anti-SOX9			1:100
Anti-Ki67	ABCAM	AB16667	1:200
Anti-LGR5	ABCAM, Sigma, Miltenyi	ab75732, HPA012530, 30-104-072	1:100
Anti-LRIG1	Invitrogen	PA5-52860	1:100

Tab.E: Antibodies for histological gastric marker validation

The secondary antibodies used were mouse or rabbit Alexa 488, 568, or 647 IgG (1:1000, Invitrogen). The primary antibodies were incubated on the slides o.n. at 4 °C. DAPI was used 1:5000 for nuclear staining. The sections were mounted in a Fluorescent Mounting Medium (Dako). Immunostainings and imaging were performed on a minimum of three biological replicates, and representative images of the replicates are included in the manuscript. Images were taken using confocal microscopy (Zeiss, LSM700). Forty biopsy samples (27 *antrum* and 13 *corpus*) derived from 35 healthy putative patients (45 - 75 years) in total were processed and analyzed. To obtain a qualitative analysis, immunostaining and imaging for each marker were performed on a minimum of three biological replicates with similar results.

H&E. H&E staining was performed on FFPE sections according to standard laboratory protocols. Briefly, five-micrometer sections from the paraffin blocks were deparaffinated, rehydrated, and washed in distilled water. Stain in Hematoxylin Solution for 15 minutes, counterstain with Eosin Y Solution, Alcoholic 5 minutes. The sections were dehydrated and mounted in Eukitt mounting medium (SIGMA).

7.9 Whole mount analysis

Organoid were collected in GF medium (Growth Factors – Medium = Advanced DMEM/F12 - 500ml, Pen/strep (100x) - 5ml, HEPES (1M=100x) - 5ml, Glutamax (100x) - 5ml). Matrigel was dissolved with Corning Cell Recovery Medium on ice for 1 h. Organoids were fixed in 4% paraformaldehyde in PBS (w/v) for 1h at RT. Organoids were permeabilized in 1% TritonX-

100 for 30 min at RT. Organoids were incubated in Blocking Buffer (protein block serum free, Dako) for 1-4h at RT. Organoids were incubated with primary antibodies o.n. at 4 °C. The secondary antibodies used were mouse or rabbit Alexa 488, 555, or 647 IgG (1:500, Invitrogen). DAPI was used at 1:5000 for nuclear staining. All steps were carried out leaving deposit organoids by gravity (5-10 min) on ice. Organoids were transferred in a small volume of 1 x PBS + 0.5% FBS on the coverslip or into a 35mm dish with a glass bottom, and they were left for 20- 30 min to sediment. The microscopic glass was left to dry at RT at least o.n. Images were taken using confocal microscopy (Zeiss, LSM700). To obtain a qualitative analysis, whole-mount immunofluorescences and imaging for each marker were performed on a minimum of three biological replicates, in cystic and complex morphology, with similar results. The processed and analyzed organoid lines are listed in **Table F.**

hGO ID
hGO#020
hGO#021
hGO#026
hGO#030
hGO#040
hGO#043

Tab.F: Human gastric organoid line used for Whole-mount immunofluorescences analysis.

7.10 Bioinformatics and HPA analysis

The principal reference of our study was the scRNASeq paper by Bussingler et al., which extensively performs a classification of the human gastrointestinal cytotypes by high-throughput sequencing technologies.

From the supplementary lists of the cited paper, we selected genes of three cytotypes (Isthmus cells, Neck cells, and Chief cells) with an adjusted p-value < 0.05 and a log2 Fold Change > 0.58.

We composed 3 Gene Expression Profile datasets of Normal Samples (GTEx, GSE13861, GSE66229), and we reported the gene Expression Level of every three lists in this composed set.

The Gene Expression Level is also reported in a numeric value to rank all possible genes more covered by the lowest z-score (chromatic scale tending towards blue) **(Tab.G)**.

The Human Protein Atlas (HPA) was used to validate bioinformatic data. A list of genes and their corresponding IHC images was searched, and localization gene expression in the isthmus, neck, and chief cells was assessed.

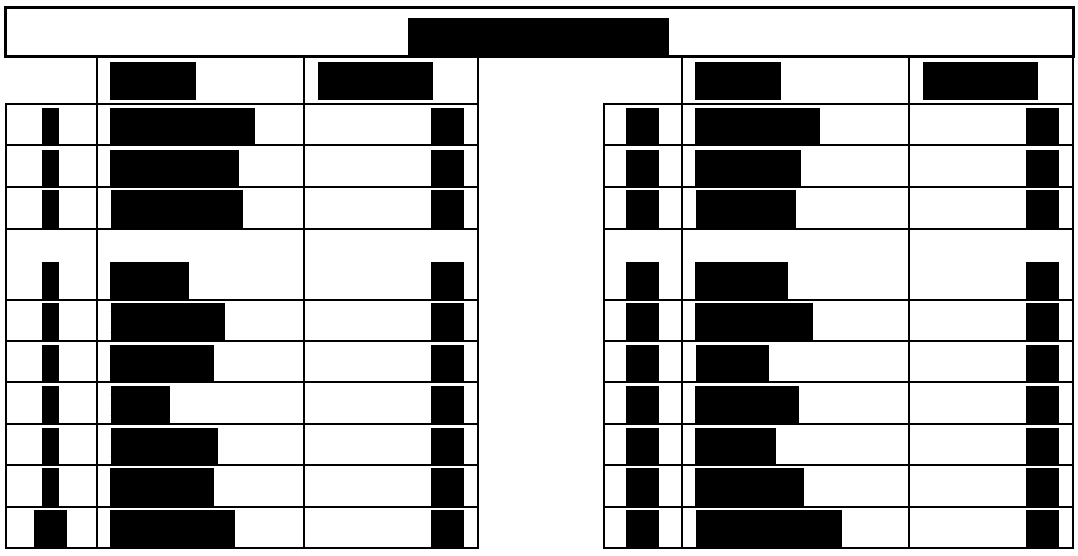
1. positive markers: protein whose expression was limited to the region of the gastric gland where the localization of the cytotype was expected;

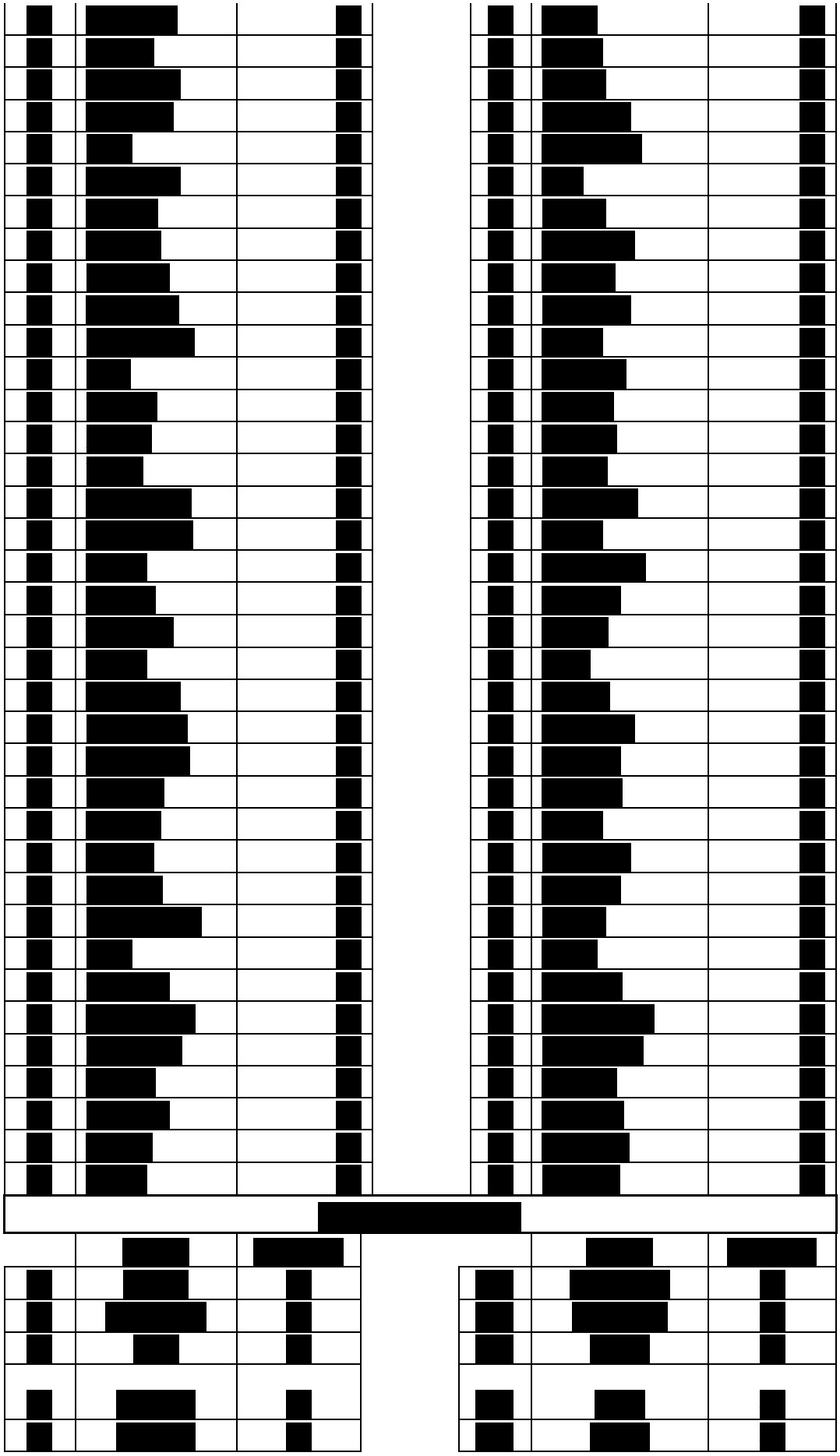
2. uninformative markers: proteins whose expression was not limited to the region of the gastric gland where the localization of the cytotype was expected;

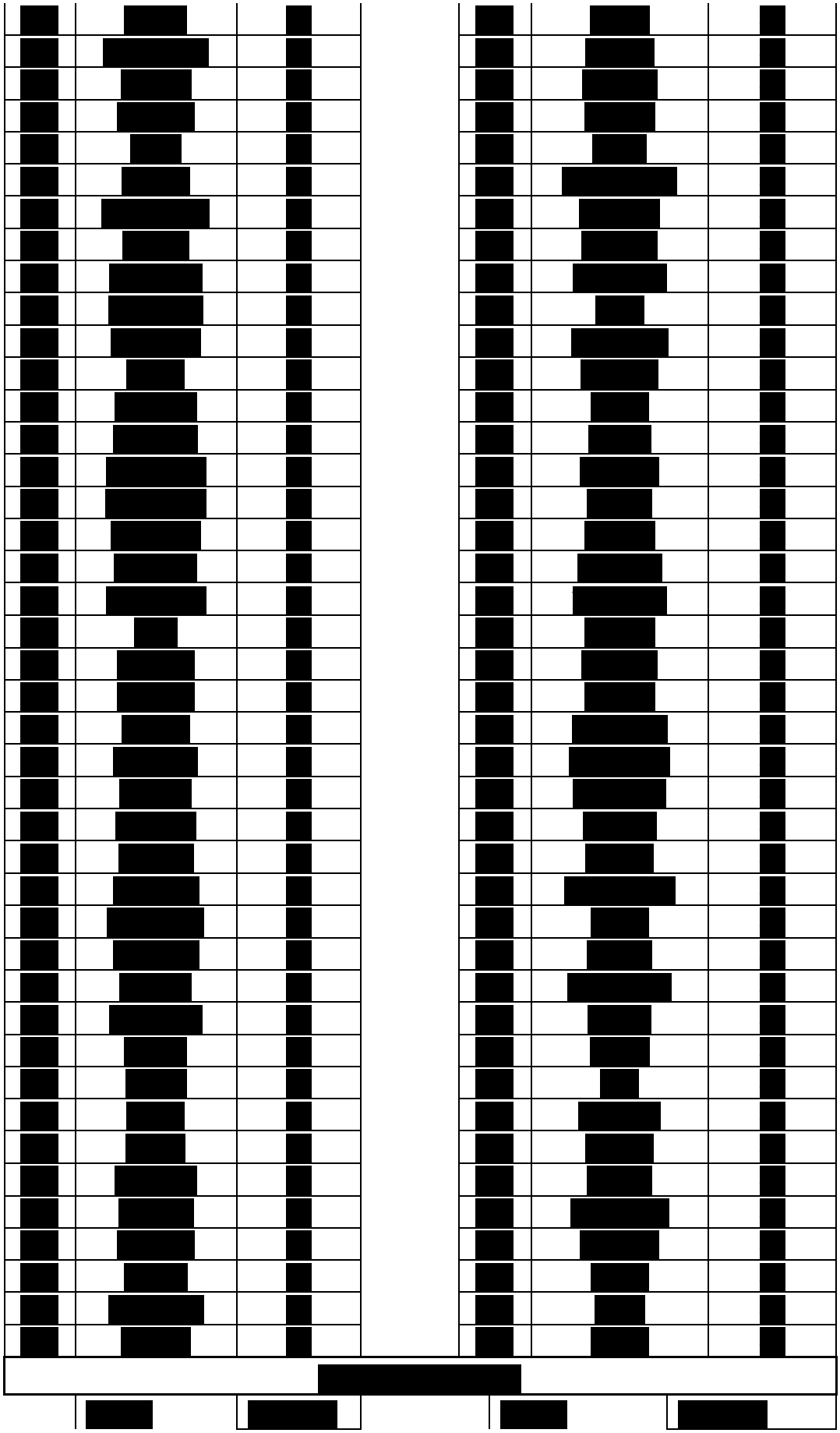
3. negative markers: proteins whose expression was not reported to the region of the gastric gland where the localization of the cytotype was expected;

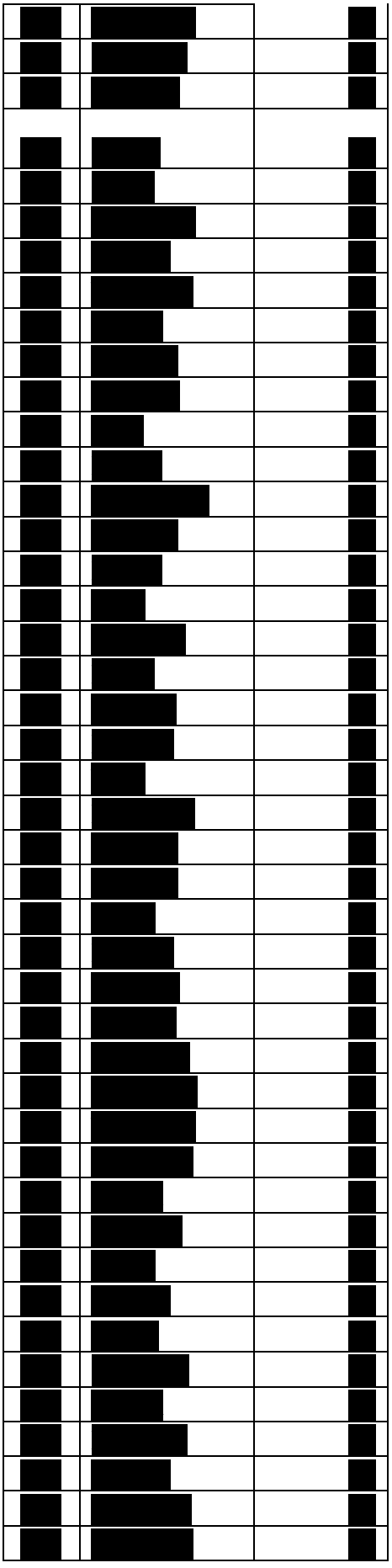
4. excluded markers: proteins whose expression was not detected on the HPA portal.

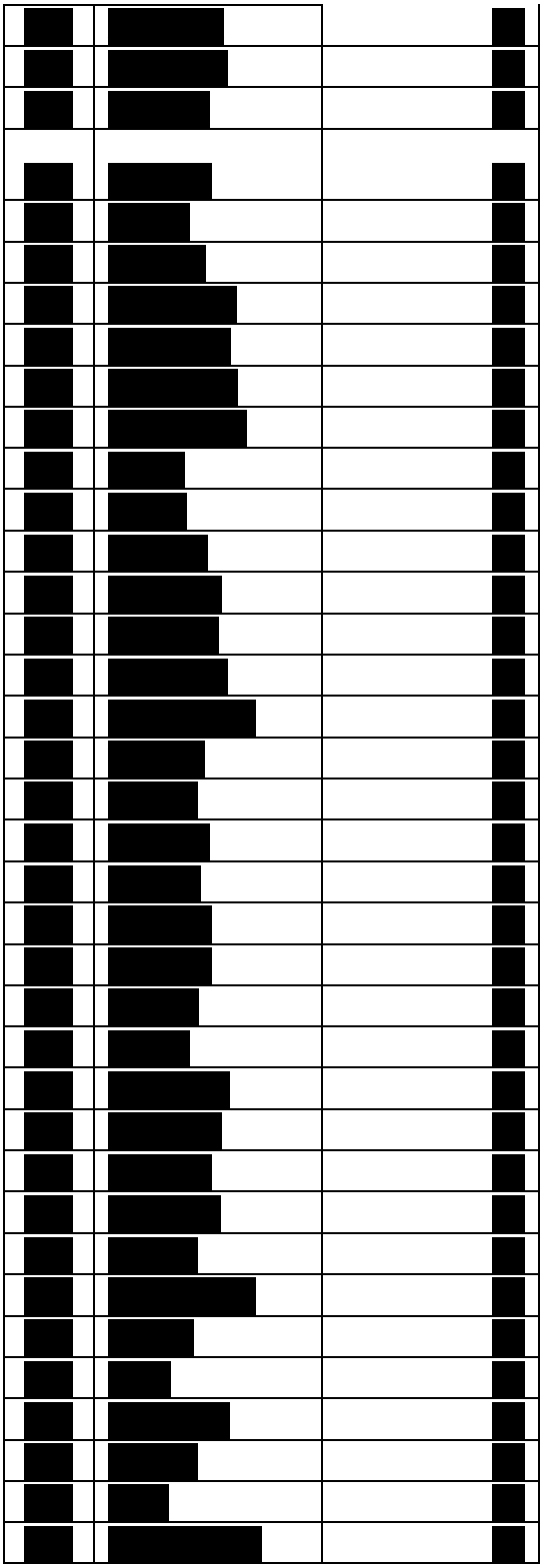
A search on PubMed (https://pubmed.ncbi.nlm.nih.gov/) and GeneCards (https://www.genecards.org/) was performed to characterize the positive markers.

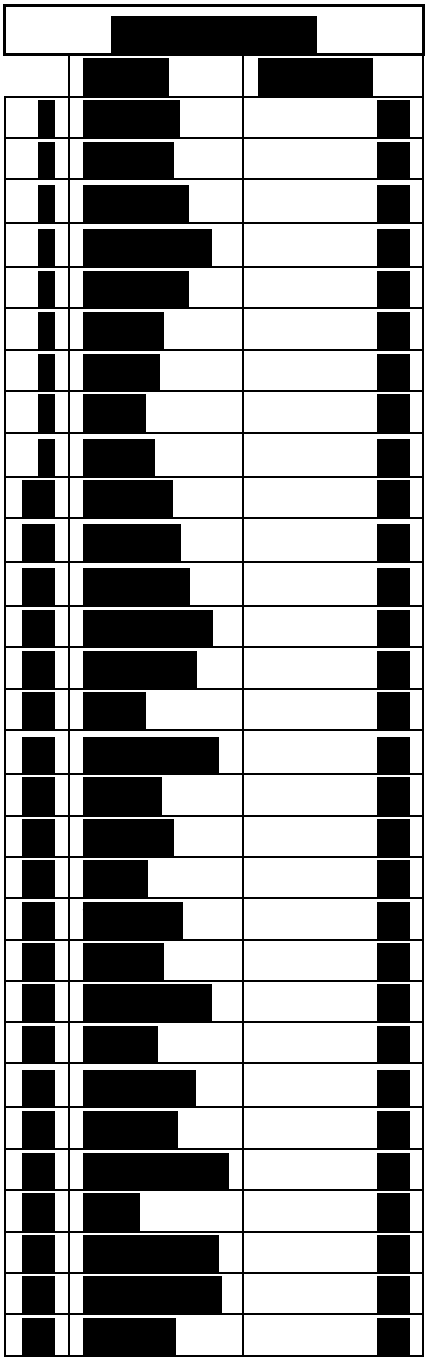


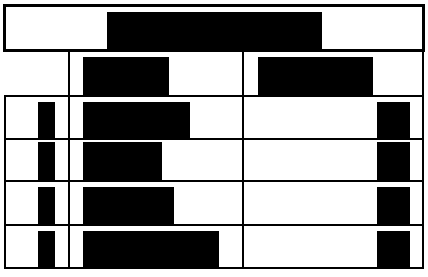


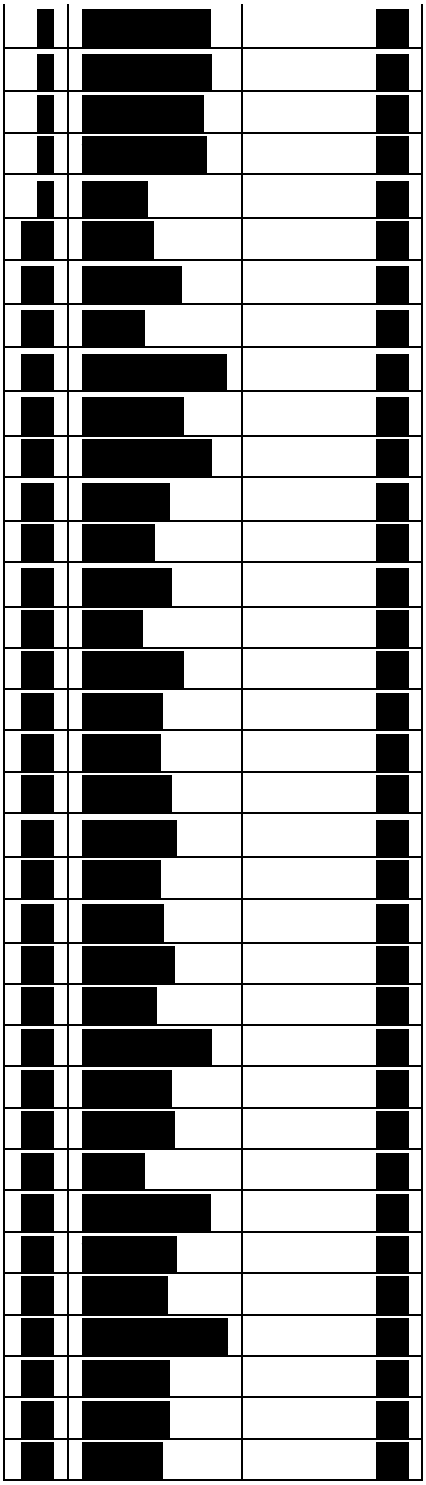












Tab.G: Specific selected genes by bioinformatics analysis. RANK1 is the number of samples that have a z-score = -1.

7.11 FACS

Single cells were sorted using BD Melody FACS. Organoids were prepared as described above. Briefly, organoids were cellularized using TrypLE express and pipetted through a p200 tip until

most cells were single. The dissociated cells were centrifuged for 5 min 1500 rpm at 4°C. The pellet was suspended in an appropriate amount of PBS 2mM EDTA. Dead cells were excluded by morphological evaluation using a gate (G1) based on the forward scatter area (FSC-A) versus the atter area (SSC-A). G1 cells were further gated to isolate single cells (G2 using FSC-A versus forward scatter height (FSC-H). G2 cells were subsequently sorted for GFP fluorescence intensity, using pLenti Cmv^{-} GFP-infected organoids as the reference signal for background fluorescence.

8. REFERENCES

1.Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019 Feb 26;10(1):68. doi: 10.1186/s13287-019-1165-5. PMID: 30808416; PMCID: PMC6390367.

2. *Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998 Nov 6 and 4, 282(5391):1145-7. doi: 10.1126/science.282.5391.1145. Erratum in: Science 1998 Dec 4;282(5395):1827. PMID: 9804556*

3. Kazutoshi Takahashi, Shinya Yamanaka, Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors, Cell, Volume 126, Issue 4, 2006, Pages 663-676, ISSN 0092-8674, https://doi.org/10.1016/j.cell.2006.07.024. Epub 2006 Aug 10. PMID: 16904174.

4. Yamanaka S. Pluripotent Stem Cell-Based Cell Therapy-Promise and Challenges. Cell Stem Cell. 2020 Oct 1;27(4):523-531. doi: 10.1016/j.stem.2020.09.014. PMID: 33007237.

5. Laurenti E, Göttgens B. From hematopoietic stem cells to complex differentiation landscapes. Nature. 2018 Jan 24;553(7689):418-426. doi: 10.1038/nature25022. PMID: 29364285; PMCID: PMC6555401.

6. Takami A. Hematopoietic stem cell transplantation for acute myeloid leukemia. Int J Hematol. 2018 May;107(5):513-518. doi: 10.1007/s12185-018-2412-8. Epub 2018 Jan 27. PMID: 29374826.

7. Clevers H. STEM CELLS. What is an adult stem cell? Science. 2015 Dec 11;350(6266):1319-20. doi: 10.1126/science.aad7016. PMID: 26659043.

8. Clevers H, Watt FM. Defining Adult Stem Cells by Function, not by Phenotype. Annu Rev Biochem. 2018 Jun 20;87:1015-1027. doi: 10.1146/annurev-biochem-062917-012341. Epub 2018 Mar 1. PMID: 29494240.

9. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H. Identification of stem cells in small intestine and

colon by marker gene Lgr5. Nature. 2007 Oct 25 and 10.1, 449(7165):1003-7. doi: 10.1038/nature06196. Epub 2007 Oct 14. PMID: 17934449.

10.Miyajima A, Tanaka M, Itoh T. Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. Cell Stem Cell. 2014 May 1;14(5):561-74. doi: 10.1016/j.stem.2014.04.010. PMID: 24792114.

11.Wang D, Cai C, Dong X, Yu QC, Zhang XO, Yang L, Zeng YA. Identification of multipotent mammary stem cells by protein C receptor expression. Nature. 2015 Jan 1;517(7532):81-4. doi: 10.1038/nature13851. Epub 2014 Oct 19. PMID: 25327250.

12.Post Y, Clevers H. Defining Adult Stem Cell Function at Its Simplest: The Ability to Replace Lost Cells through Mitosis. Cell Stem Cell. 2019 Aug 1;25(2):174-183. doi: 10.1016/j.stem.2019.07.002. PMID: 31374197.

13. Andersson-Rolf A, Zilbauer M, Koo BK, Clevers H. Stem Cells in Repair of Gastrointestinal Epithelia. Physiology (Bethesda). 2017 Jul;32(4):278-289. doi: 10.1152/physiol.00005.2017. PMID: 28615312; PMCID: PMC5545610.

14. Bartfeld S, Koo BK. Adult gastric stem cells and their niches. Wiley Interdiscip Rev Dev Biol. 2017 Mar and 28044412., 6(2). doi: 10.1002/wdev.261. Epub 2017 Jan 3. PMID: 28044412.

15. Soybel DI. Anatomy and physiology of the stomach. Surg Clin North Am. 2005 Oct;85(5):875-94, v. doi: 10.1016/j.suc.2005.05.009. PMID: 16139026.

16. Choi E, Roland JT, Barlow BJ, O'Neal R, Rich AE, Nam KT, Shi C, Goldenring JR. Cell lineage distribution atlas of the human stomach reveals heterogeneous gland populations in the gastric antrum. Gut. 2014 Nov;63(11):1711-20. doi: 10.1136/gutjnl-2013-305964. Epub 2014 Jan 31. PMID: 24488499; PMCID: PMC4117823.

17. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. Science. 2010 Jan 29, 20110496, 327(5965):542-5. doi: 10.1126/science.1180794. PMID: and PMC4105182.

18. Hicks MR, Pyle AD. The emergence of the stem cell niche. Trends Cell Biol. 2023 Feb;33(2):112-123. doi: 10.1016/j.tcb.2022.07.003. Epub 2022 Aug 4. PMID: 35934562; PMCID: PMC9868094

19. Ferraro F, Celso CL, Scadden D. Adult stem cells and their niches. Adv Exp Med Biol. 2010, 21222205, 695:155-68. doi: 10.1007/978-1-4419-7037-4_11. PMID: and PMC4020242.

20. van den Brink GR, Hardwick JC, Tytgat GN, Brink MA, Ten Kate FJ, Van Deventer SJ, Peppelenbosch MP. Sonic hedgehog regulates gastric gland morphogenesis in man and mouse. Gastroenterology. 2001 Aug and 114875, 121(2):317-28. doi: 10.1053/gast.2001.26261. PMID: 11487541.

21. Kim TH, Shivdasani RA. Notch signaling in stomach epithelial stem cell homeostasis. J Exp Med. 2011 Apr 11, 21402740, 208(4):677-88. doi: 10.1084/jem.20101737. Epub 2011 Mar 14. PMID: and PMC3137787.

22. Demitrack ES, Gifford GB, Keeley TM, Carulli AJ, VanDussen KL, Thomas D, Giordano TJ, Liu Z, Kopan R, Samuelson LC. Notch signaling regulates gastric antral LGR5 stem cell function. EMBO J. 2015 Oct 14 and 20, 34(20):2522-36. doi: 10.15252/embj.201490583. Epub 2015 Aug 12. PMID: 26271103; PMCID: PMC4609184.
23. Spencer-Dene B, Sala FG, Bellusci S, Gschmeissner S, Stamp G, Dickson C. Stomach development is dependent on fibroblast growth factor 10/fibroblast growth factor receptor 2b-mediated signaling. Gastroenterology. 2006 Apr and 10.1053/j.gas, 130(4):1233-44. doi: 10.1053/j.gastro.2006.02.018. PMID: 16618415.

24. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M. et al. Lgr5+ve stem cells drive self- renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 2010, 6:25–36. doi: 10.1016/j.stem.2009.11.013. PMID: 20085740.

25. Dempsey PJ, Goldenring JR, Soroka CJ, Modlin IM, McClure RW, Lind CD, Ahlquist DA, Pittelkow MR, Lee DC, Sandgren EP, et al. Possible role of transforming growth factor alpha in the pathogenesis of Ménétrier's disease: supportive evidence form humans and and transgenic mice. Gastroenterology. 1992 Dec;103(6):1950-63. doi: 10.1016/0016-5085(92)91455-d. PMID: 1451986.

26. Kelly EJ, Newell SJ, Brownlee KG, Farmery SM, Cullinane C, Reid WA, Jackson P, Gray SF, Primrose JN, Lagopoulos M. Role of epidermal growth factor and transforming growth factor alpha in the developing stomach. Arch Dis Child Fetal Neonatal Ed. 1997 May;76(3):F158-62. doi: 10.1136/fn.76.3.f158. Erratum in: Arch Dis Child Fetal Neonatal Ed 1997 Sep;77(2):F159. PMID: 9175944; PMCID: PMC1720655.

27. Hayakawa Y, Jin G, Wang H, Chen X, Westphalen CB, Asfaha S, Renz BW, Ariyama H, Dubeykovskaya ZA, Takemoto Y, Lee Y, Muley A, Tailor Y, Chen D, Muthupalani S, Fox JG, Shulkes A, Worthley DL, Takaishi S, Wang TC. CCK2R identifies and regulates gastric antral stem cell states and carcinogenesis. Gut. 2015 Apr;64(4):544-53. doi: 10.1136/gutjnl-2014-307190. Epub 2014 Jun 20. PMID: 24951258; PMCID: PMC4627594.

28. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M, Stange DE, van Es JE, Guardavaccaro D, Schasfoort RB, Mohri Y, Nishimori K, Mohammed S, Heck AJ, Clevers H. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature. 2011 Jul 4;476(7360):293-7. doi: 10.1038/nature10337. PMID: 21727895.

29. Koo BK, Spit M, Jordens I, Low TY, Stange DE, van de Wetering M, van Es JH, Mohammed S, Heck AJ, Maurice MM, Clevers H. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. Nature. 2012 Aug 30 and 1, 488(7413):665-9. doi: 10.1038/nature11308. PMID: 22895187.

30. Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgård R. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet. 2008 Nov and 18849992., 40(11):1291-9. doi: 10.1038/ng.239. Epub 2008 Oct 12. PMID: 18849992.

31. Huch M, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, Sojoodi M, Li VS, Schuijers J, Gracanin A, Ringnalda F, Begthel H, Hamer K, Mulder J, van Es JH, de Koning E, Vries RG, Heimberg H, Clevers H. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. EMBO J. 2013 Oct 16;32(20):2708-21. doi: 10.1038/emboj.2013.204. Epub 2013 Sep 17. PMID: 24045232; PMCID: PMC3801438.

32. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, Dowling CM, Gao D, Begthel H, Sachs N, Vries RGJ, Cuppen E, Chen Y, Sawyers CL, Clevers HC. Identification of multipotent luminal progenitor cells in human prostate organoid culture. Cell. 2014 Sep 25;159(1):163-175. doi: 10.1016/j.cell.2014.08.017. Epub 2014 Sep 4. PMID: 25201529; PMCID: PMC4772677.

33. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ, Haft A, Vries RG, Grompe M, Clevers H. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature. 2013 Feb 14 and 494 (7436):247-50. doi: 10.1038/nature11826. Epub 2013 Jan 27. PMID: 23354049; PMCID: PMC3634804.

34. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, Ellis E, van Wenum M, Fuchs SA, de Ligt J, van de Wetering M, Sasaki N, Boers SJ, Kemperman H, de Jonge J, Ijzermans JN, Nieuwenhuis EE, Hoekstra R, Strom S, Vries RR, van der Laan LJ, Cuppen E, Clevers H. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015 Jan 15;160(1-2):299-312. doi: 10.1016/j.cell.2014.11.050. Epub 2014 Dec 18. PMID: 25533785; PMCID: PMC4313365.

35. Stange DE, Koo BK, Huch M, Sibbel G, Basak O, Lyubimova A, Kujala P, Bartfeld S, Koster J, Geahlen JH, Peters PJ, van Es JH, van de Wetering M, Mills JC, Clevers H. Differentiated Troy+ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. Cell. 2013 Oct 10;155(2):357-68. doi: 10.1016/j.cell.2013.09.008. PMID: 24120136; PMCID: PMC4094146.

36. Nam KT, Lee HJ, Sousa JF, Weis VG, O'Neal RL, Finke PE, Romero-Gallo J, Shi G, Mills JC, Peek RM Jr, Konieczny SF, Goldenring JR. Mature chief cells are cryptic progenitors for metaplasia in the stomach. Gastroenterology. 2010 Dec and doi, 139(6):2028-2037.e9. doi: 10.1053/j.gastro.2010.09.005. Epub 2010 Sep 18. PMID: 20854822; PMCID: PMC2997152.

37. Qiao XT, Ziel JW, McKimpson W, Madison BB, Todisco A, Merchant JL, Samuelson LC, Gumucio DL. Prospective identification of a multilineage progenitor in murine stomach epithelium. Gastroenterology. 2007 Dec and 10.1053/j.gastro.2007.09.031, 133(6):1989-98. doi: 10.1053/j.gastro.2007.09.031. PMID: 18054570; PMCID: PMC2329573.

38. Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, Sengupta S, Seandel M, Geijsen N, Hochedlinger K. Sox2(+) adult stem and progenitor cells are important for tissue regeneration and survival of mice. Cell Stem Cell. 2011 Oct 4 and 10.1016/j.s, 9(4):317-29. doi: 10.1016/j.stem.2011.09.001. PMID: 21982232; PMCID: PMC3538360.

39. Sigal M, Logan CY, Kapalczynska M, Mollenkopf HJ, Berger H, Wiedenmann B, Nusse R, Amieva MR, Meyer TF. Stromal R-spondin orchestrates gastric epithelial stem cells and

gland homeostasis. Nature. 2017 Aug 24 and Ep, 548(7668):451-455. doi: 10.1038/nature23642. Epub 2017 Aug 16. PMID: 28813421.

40. Zhao R, He B, Bie Q, Cao J, Lu H, Zhang Z, Liang J, Wei L, Xiong H, Zhang B. AQP5 complements LGR5 to determine the fates of gastric cancer stem cells through regulating ULK1 ubiquitination. J Exp Clin Cancer Res. 2022 Nov 14 and 10.1186/s1304, 41(1):322. doi: 10.1186/s13046-022-02532-w. Erratum in: J Exp Clin Cancer Res. 2022 Dec 13;41(1):342. PMID: 36372898; PMCID: PMC9661769.

41. Tan SH, Swathi Y, Tan S, Goh J, Seishima R, Murakami K, Oshima M, Tsuji T, Phuah P, Tan LT, Wong E, Fatehullah A, Sheng T, Ho SWT, Grabsch HI, Srivastava S, Teh M, Denil SLIJ, Mustafah S, Tan P, Shabbir A, So J, Yeoh KG, Barker N. AQP5 enriches for stem cells and cancer origins in the distal stomach. Nature. 2020 Feb;578(7795):437-443. doi: 10.1038/s41586-020-1973-x. Epub 2020 Feb 5. PMID: 32025032.

42. Quante M, Marrache F, Goldenring JR, Wang TC. TFF2 mRNA transcript expression marks a gland progenitor cell of the gastric oxyntic mucosa. Gastroenterology. 2010 Dec and 20708616, 139(6):2018-2027.e2. doi: 10.1053/j.gastro.2010.08.003. Epub 2010 Aug 12. PMID: 20708616; PMCID: PMC2997174.

43. Farrell JJ, Taupin D, Koh TJ, Chen D, Zhao CM, Podolsky DK, Wang TC. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. J Clin Invest. 2002 Jan and 10.1172/, 109(2):193-204. doi: 10.1172/JCI12529. PMID: 11805131; PMCID: PMC150833.

44. Nienhüser H, Kim W, Malagola E, Ruan T, Valenti G, Middelhoff M, Bass A, Der CJ, Hayakawa Y, Wang TC. Mist1+ gastric isthmus stem cells are regulated by Wnt5a and expand in response to injury and inflammation in mice. Gut. 2021 Apr and 10., 70(4):654-665. doi: 10.1136/gutjnl-2020-320742. Epub 2020 Jul 24. PMID: 32709613; PMCID: PMC10337481.

45. Choi E, Lantz TL, Vlacich G, Keeley TM, Samuelson LC, Coffey RJ, Goldenring JR, Powell AE. Lrig1+ gastric isthmal progenitor cells restore normal gastric lineage cells during damage recovery in adult mouse stomach. Gut. 2018 Sep and 10.1, 67(9):1595-1605. doi: 10.1136/gutjnl-2017-313874. Epub 2017 Aug 16. PMID: 28814482; PMCID: PMC5815959.

46. Matsuo J, Kimura S, Yamamura A, Koh CP, Hossain MZ, Heng DL, Kohu K, Voon DC, Hiai H, Unno M, So JB, Zhu F, Srivastava S, Teh M, Yeoh KG, Osato M, Ito Y. Identification of Stem Cells in the Epithelium of the Stomach Corpus and Antrum of Mice. Gastroenterology. 2017 Jan;152(1):218-231.e14. doi: 10.1053/j.gastro.2016.09.018. Epub 2016 Sep 23. PMID: 27670082.

47. Yoshioka T, Fukuda A, Araki O, Ogawa S, Hanyu Y, Matsumoto Y, Yamaga Y, Nakanishi Y, Kawada K, Sakai Y, Chiba T, Seno H. Bmi1 marks gastric stem cells located in the isthmus in mice. J Pathol. 2019 Jun and Feb, 248(2):179-190. doi: 10.1002/path.5244. Epub 2019 Feb 20. PMID: 30689202.

48. Tsubosaka A, Komura D, Kakiuchi M, Katoh H, Onoyama T, Yamamoto A, Abe H, Seto Y, Ushiku T, Ishikawa S. Stomach encyclopedia: Combined single-cell and spatial

transcriptomics reveal cell diversity and homeostatic regulation of human stomach. Cell Rep. 2023 Oct 31;42(10):113236. doi: 10.1016/j.celrep.2023.113236. Epub 2023 Oct 17. PMID: 37819756.

49. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. Science. 2014 Jul 18 and 25035496., 345(6194):1247125. doi: 10.1126/science.1247125. Epub 2014 Jul 17. PMID: 25035496.

50. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P, Vries R, Peters PJ, Clevers H. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology. 2015 Jan and 1, 148(1):126-136.e6. doi: 10.1053/j.gastro.2014.09.042. Epub 2014 Oct 13. PMID: 25307862; PMCID: PMC4274199.

51. Lugli N, Kamileri I, Keogh A, Malinka T, Sarris ME, Talianidis I, Schaad O, Candinas D, Stroka D, Halazonetis TD. R-spondin 1 and noggin facilitate expansion of resident stem cells from non-damaged gallbladders. EMBO Rep. 2016 May and 10.15, 17(5):769-79. doi: 10.15252/embr.201642169. Epub 2016 Mar 18. PMID: 26993089; PMCID: PMC5341509.

52. Boj SF, Hwang CI, Baker LA, Chio II, Engle DD, Corbo V, Jager M, Ponz-Sarvise M, Tiriac H, Spector MS, Gracanin A, Oni T, Yu KH, van Boxtel R, Huch M, Rivera KD, Wilson JP, Feigin ME, Öhlund D, Handly-Santana A, Ardito-Abraham CM, Ludwig M, Elyada E, Alagesan B, Biffi G, Yordanov GN, Delcuze B, Creighton B, Wright K, Park Y, Morsink FH, Molenaar IQ, Borel Rinkes IH, Cuppen E, Hao Y, Jin Y, Nijman IJ, Iacobuzio-Donahue C, Leach SD, Pappin DJ, Hammell M, Klimstra DS, Basturk O, Hruban RH, Offerhaus GJ, Vries RG, Clevers H, Tuveson DA. Organoid models of human and mouse ductal pancreatic cancer. Cell. 2015 Jan 15;160(1-2):324-38. doi: 10.1016/j.cell.2014.12.021. Epub 2014 Dec 31. PMID: 25557080; PMCID: PMC4334572.

53. DeWard AD, Cramer J, Lagasse E. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. Cell Rep. 2014 Oct 23 and 25373907, 9(2):701-11. doi: 10.1016/j.celrep.2014.09.027. Epub 2014 Oct 16. PMID: 25373907; PMCID: PMC4223874.

54. Kessler M, Hoffmann K, Brinkmann V, Thieck O, Jackisch S, Toelle B, Berger H, Mollenkopf HJ, Mangler M, Sehouli J, Fotopoulou C, Meyer TF. The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. Nat Commun. 2015. Dec 8;6:8989. doi: 10.1038/ncomms9989. PMID: 26643275; PMCID: PMC4686873.

55. Zhang L, Adileh M, Martin ML, Klingler S, White J, Ma X, Howe LR, Brown AM, Kolesnick R. Establishing estrogen-responsive mouse mammary organoids from single Lgr5+ cells. Cell Signal. 2017 Jan and 7., 29:41-51. doi: 10.1016/j.cellsig.2016.08.001. Epub 2016 Aug. 7. PMID: 27511963; PMCID: PMC5138122.

56. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL. Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc Natl Acad Sci U S A. 2009 Aug 4 and 22., 106(31):12771-5. doi: 10.1073/pnas.0906850106. Epub 2009 Jul. 22. PMID: 19625615; PMCID: PMC2714281.

57. Chua CW, Shibata M, Lei M, Toivanen R, Barlow LJ, Bergren SK, Badani KK, McKiernan JM, Benson MC, Hibshoosh H, Shen MM. Single luminal epithelial progenitors can generate prostate organoids in culture. Nat Cell Biol. 2014 Oct and 16(10):951-61, 1-4. doi: 10.1038/ncb3047. Epub 2014 Sep 21. PMID: 25241035; PMCID: PMC4183706.

58. Nanduri LS, Baanstra M, Faber H, Rocchi C, Zwart E, de Haan G, van Os R, Coppes RP. Purification and ex vivo expansion of fully functional salivary gland stem cells. Stem Cell Reports. 2014 Dec 9 and O, 3(6):957-64. doi: 10.1016/j.stemcr.2014.09.015. Epub 2014 Oct 23. PMID: 25448065; PMCID: PMC4264052.

59. Ren W, Lewandowski BC, Watson J, Aihara E, Iwatsuki K, Bachmanov AA, Margolskee RF, Jiang P. Single Lgr5- or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. Proc Natl Acad Sci U S A. 2014 Nov 18 and 10.1073/, 111(46):16401-6. doi: 10.1073/pnas.1409064111. Epub 2014 Nov 3. PMID: 25368147; PMCID: PMC4246268.

60. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA. Cerebral organoids model human brain development and microcephaly. Nature. 2013 Sep 19 and 2, 501(7467):373-9. doi: 10.1038/nature12517. Epub. 2013 Aug 28. PMID: 23995685; PMCID: PMC3817409.

61. Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, Saito K, Yonemura S, Eiraku M, Sasai Y. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell. 2012 Jun 14 and 10.1016/j.stem.2012., 10(6):771-785. doi: 10.1016/j.stem.2012.05.009. PMID: 22704518.

62. Clevers H, Loh KM, Nusse R. Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science. 2014 Oct 3 and 25278615., 346(6205):1248012. doi: 10.1126/science.1248012. Epub 2014 Oct 2. PMID: 25278615.

63. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK, Nieuwenhuis EE, Beekman JM, Clevers H. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell. 2013 Dec 5;13(6):653-8. doi: 10.1016/j.stem.2013.11.002. PMID: 24315439.

64. Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, Ichinose S, Nagaishi T, Okamoto R, Tsuchiya K, Clevers H, Watanabe M. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5^{+} stem cell. Nat Med. 2012 Mar 11;18(4):618-23. doi: 10.1038/nm.2695. PMID: 22406745.

65. Drost J, Clevers H. Organoids in cancer research. Nat Rev Cancer. 2018 Jul and 29692415., 18(7):407-418. doi: 10.1038/s41568-018-0007-6. PMID: 29692415.

66. Busslinger GA, Weusten BLA, Bogte A, Begthel H, Brosens LAA, Clevers H. Human gastrointestinal epithelia of the esophagus, stomach, and duodenum resolved at single-cell resolution. Cell Rep. 2021 Mar 9 and PMI, 34(10):108819. doi: 10.1016/j.celrep.2021.108819. PMID: 33691112.

67. Shi D, Xi XX. Regulation of MUC6 Methylation Correlates with Progression of Gastric Cancer. Yonsei Med J. 2021 Nov, 34672134, 62(11):1005-1015. doi: 10.3349/ymj.2021.62.11.1005. PMID: 34672134; PMCID: PMC8542475.

68. Yamanoi K, Nakayama J. Reduced αGlcNAc glycosylation on gastric gland mucin is a biomarker of malignant potential for gastric cancer, Barrett's adenocarcinoma, and pancreatic cancer. Histochem Cell Biol. 2018 Jun;149(6):569-575. doi: 10.1007/s00418-018-1667-8. Epub 2018 Apr 16. PMID: 29658052.

69. Heuer F, Stürmer R, Heuer J, Kalinski T, Lemke A, Meyer F, Hoffmann W. Different Forms of TFF2, A Lectin of the Human Gastric Mucus Barrier: In Vitro Binding Studies. Int J Mol Sci. 2019 Nov 22, 31771101, 20(23):5871. doi: 10.3390/ijms20235871. doi: 10.3390/ijms20235871. PMID: 31771101; PMCID: PMC6928932.

70. Kong Y, Zheng Y, Jia Y, Li P, Wang Y. Decreased LIPF expression is correlated with DGKA and predicts poor outcome of gastric cancer. Oncol Rep. 2016 Oct, 27498782, 36(4):1852-60. doi: 10.3892/or.2016.4989. Epub 2016 Aug 1. PMID: 27498782; PMCID: PMC5022960.

71. Shen S, Jiang J, Yuan Y. Pepsinogen C expression, regulation and its relationship with cancer. Cancer Cell Int. 2017 May 23, 28546787, 17:57. doi: 10.1186/s12935-017-0426-6. PMID: 28546787; PMCID: PMC5442862.

72. Caldwell B, Meyer AR, Weis JA, Engevik AC, Choi E. Chief cell plasticity is the origin of metaplasia following acute injury in the stomach mucosa. Gut. 2022 Jun, 34497145, 71(6):1068-1077. doi: 10.1136/gutjnl-2021-325310. Epub 2021 Sep 8. PMID: 34497145; PMCID: PMC8901801.

73. Uchi R, Kogo R, Kawahara K, Sudo T, Yokobori T, Eguchi H, Sugimachi K, Maehama T, Mori M, Suzuki A, Komune S, Mimori K. PICT1 regulates TP53 via RPL11 and is involved in gastric cancer progression. Br J Cancer. 2013 Oct 15 and 10.1038/bj, 109(8):2199-206. doi: 10.1038/bjc.2013.561. Epub 2013 Sep 17. PMID: 24045667; PMCID: PMC3798961.

74. Guo Y, Shi J, Zhang J, Li H, Liu B, Guo H. Polypeptide N-acetylgalactosaminyltransferase-6 expression in gastric cancer. Onco Targets Ther. 2017 Jul 7, 28744137, 10:3337-3344. doi: 10.2147/OTT.S138590. PMID: 28744137; PMCID: PMC5511018.

75. Cao L, Chen Y, Xu S, Cheng H. Is NAD(P)H quinone oxidoreductase 1 a tumor promoter or suppressor in gastric cancer? Front Oncol. 2023 Apr 28, 37188198, 13:1143108. doi: 10.3389/fonc.2023.1143108. PMID: and PMC10178065. PMID: 37188198; PMCID: PMC10178065.

76. Ning S, Wei W, Li J, Hou B, Zhong J, Xie Y, Liu H, Mo X, Chen J, Zhang L. Clinical significance and diagnostic capacity of serum TK1, CEA, CA 19-9 and CA 72-4 levels in gastric and colorectal cancer patients. J Cancer. 2018 Jan 1 and 10.715, 9(3):494-501. doi: 10.7150/jca.21562. PMID: 29483954; PMCID: PMC5820916.

77. Lee HJ, Nam KT, Park HS, Kim MA, Lafleur BJ, Aburatani H, Yang HK, Kim WH, Goldenring JR. Gene expression profiling of metaplastic lineages identifies CDH17 as a prognostic marker in early stage gastric cancer. Gastroenterology. 2010 Jul and 139(1):213-25.e3. doi: 10.1053/j.gastro.2010.04.008. Epub 2010 Apr 13. PMID: 20398667; PMCID: PMC2917327.

78. Claerhout S, Lim JY, Choi W, Park YY, Kim K, Kim SB, Lee JS, Mills GB, Cho JY. Gene expression signature analysis identifies vorinostat as a candidate therapy for gastric cancer. PLoS One. 2011 and Sep, 6(9):e24662. doi: 10.1371/journal.pone.0024662. Epub 2011 Sep 9. PMID: 21931799; PMCID: PMC3170379.

79. Cho CJ, Park D, Mills JC. ELAPOR1 is a secretory granule maturation-promoting factor that is lost during paligenosis. Am J Physiol Gastrointest Liver Physiol. 2022 Jan 1, 34816763, 322(1):G49-G65. doi: 10.1152/ajpgi.00246.2021. Epub 2021 Nov 24. PMID: 34816763; PMCID: PMC8698547.

80. Cui J, Li F, Wang G, Fang X, Puett JD, Xu Y. Gene-expression signatures can distinguish gastric cancer grades and stages. PLoS One. 2011 Mar 18, 21445269, 6(3):e17819. doi: 10.1371/journal.pone.0017819. PMID: 21445269; PMCID: PMC3060867.

81. Xu XY, Zhang LJ, Yu YQ, Zhang XT, Huang WJ, Nie XC, Song GQ. Down-regulated MAC30 expression inhibits proliferation and mobility of human gastric cancer cells. Cell Physiol Biochem. 2014 and 248532, 33(5):1359-68. doi: 10.1159/000358703. Epub 2014 May 5. PMID: 24853233.

82. Wu X, Zhou F, Ji X, Ren K, Shan Y, Mao X, Fen Y, Chen R, Ding H, Fu X. The prognostic role of MAC30 in advanced gastric cancer patients receiving platinum-based chemotherapy. Future Oncol. 2017 Dec and O, 13(29):2691-2696. doi: 10.2217/fon-2017-0426. Epub 2017 Oct 3. PMID: 28972404.

83. Wang W, He Y, Zhao Q, Zhao X, Li Z. Identification of potential key genes in gastric cancer using bioinformatics analysis. Biomed Rep. 2020 Apr, 32190306, 12(4):178-192. doi: 10.3892/br.2020.1281. Epub 2020 Feb 20. PMID: 32190306; PMCID: PMC7054703.

84. Ariyachet C, Tovaglieri A, Xiang G, Lu J, Shah MS, Richmond CA, Verbeke C, Melton DA, Stanger BZ, Mooney D, Shivdasani RA, Mahony S, Xia Q, Breault DT, Zhou Q. Reprogrammed Stomach Tissue as a Renewable Source of Functional β Cells for Blood Glucose Regulation. Cell Stem Cell. 2016 Mar 3;18(3):410-21. doi: 10.1016/j.stem.2016.01.003. Epub 2016 Feb 18. PMID: 26908146; PMCID: PMC4779391.

85. Mihaylova MM, Cheng CW, Cao AQ, Tripathi S, Mana MD, Bauer-Rowe KE, Abu-Remaileh M, Clavain L, Erdemir A, Lewis CA, Freinkman E, Dickey AS, La Spada AR, Huang Y, Bell GW, Deshpande V, Carmeliet P, Katajisto P, Sabatini DM, Yilmaz ÖH. Fasting Activates Fatty Acid Oxidation to Enhance Intestinal Stem Cell Function during Homeostasis and Aging. Cell Stem Cell. 2018 May 3;22(5):769-778.e4. doi: 10.1016/j.stem.2018.04.001. PMID: 29727683; PMCID: PMC5940005.

86. Nalapareddy K, Nattamai KJ, Kumar RS, Karns R, Wikenheiser-Brokamp KA, Sampson LL, Mahe MM, Sundaram N, Yacyshyn MB, Yacyshyn B, Helmrath MA, Zheng Y, Geiger H. Canonical Wnt Signaling Ameliorates Aging of Intestinal Stem Cells. Cell Rep. 2017 Mar 14 ;18(11):2608-2621. doi: 10.1016/j.celrep.2017.02.056. PMID: 28297666; PMCID: PMC5987258.

87. Asano N, Takeuchi A, Imatani A, Saito M, Jin X, Hatta W, Uno K, Koike T, Masamune A. Wnt Signaling and Aging of the Gastrointestinal Tract. Int J Mol Sci. 2022 Oct 13, 36293064,

23(20):12210. doi: 10.3390/ijms232012210. doi: 10.3390/ijms232012210. PMID: 36293064; PMCID: PMC9603545.

88. Schweiger PJ, Clement DL, Page ME, Schepeler T, Zou X, Sirokmány G, Watt FM, Jensen KB. Lrig1 marks a population of gastric epithelial cells capable of long-term tissue maintenance and growth in vitro. Sci Rep. 2018 Oct 15 and 10.1038/s41598-, 8(1):15255. doi: 10.1038/s41598-018-33578-6. PMID: 30323305; PMCID: PMC6189208.

89. Xiao S, Zhou L. Gastric Stem Cells: Physiological and Pathological Perspectives. Front Cell Dev Biol. 2020 Sep 17, 33043003, 8:571536. doi: 10.3389/fcell.2020.571536. PMID: 33043003; PMCID: PMC7527738.

90. Kashyap V, Rezende NC, Scotland KB, Shaffer SM, Persson JL, Gudas LJ, Mongan NP. Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. Stem Cells Dev. 2009 Sep;18(7):1093-108. doi: 10.1089/scd.2009.0113. PMID: 19480567; PMCID: PMC3135180.

91. Blache P, van de Wetering M, Duluc I, Domon C, Berta P, Freund JN, Clevers H, Jay P. SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes. J Cell Biol. 2004 Jul 5 and 10.108, 166(1):37-47. doi: 10.1083/jcb.200311021. PMID: 15240568; PMCID: PMC2172132.

92. Santos JC, Carrasco-Garcia E, Garcia-Puga M, Aldaz P, Montes M, Fernandez-Reyes M, de Oliveira CC, Lawrie CH, Araúzo-Bravo MJ, Ribeiro ML, Matheu A. SOX9 Elevation Acts with Canonical WNT Signaling to Drive Gastric Cancer Progression. Cancer Res. 2016 Nov. 15;76(22):6735-6746. doi: 10.1158/0008-5472.CAN-16-1120. Epub 2016 Aug 28. PMID: 27569216.

93. Khurana SS, Riehl TE, Moore BD, Fassan M, Rugge M, Romero-Gallo J, Noto J, Peek RM Jr, Stenson WF, Mills JC. The hyaluronic acid receptor CD44 coordinates normal and metaplastic gastric epithelial progenitor cell proliferation. J Biol Chem. 2013 May 31;288(22):16085-97. doi: 10.1074/jbc.M112.445551. Epub 2013 Apr 15. PMID: 23589310; PMCID: PMC3668764.

94. Bertaux-Skeirik N, Wunderlich M, Teal E, Chakrabarti J, Biesiada J, Mahe M, Sundaram N, Gabre J, Hawkins J, Jian G, Engevik AC, Yang L, Wang J, Goldenring JR, Qualls JE, Medvedovic M, Helmrath MA, Diwan T, Mulloy JC, Zavros Y. CD44 variant isoform 9 emerges in response to injury and contributes to the regeneration of the gastric epithelium. J Pathol. 2017 Aug;242(4):463-475. doi: 10.1002/path.4918. PMID: 28497484; PMCID: PMC5591948.

95. Zhao P, Li Y, Lu Y. Aberrant expression of CD133 protein correlates with Ki-67 expression and is a prognostic marker in gastric adenocarcinoma. BMC Cancer. 2010 May 20, 20487522, 10:218. doi: 10.1186/1471-2407-10-218. PMID: 20487522; PMCID: PMC2891633.

96. Wu C, Xie Y, Gao F, Wang Y, Guo Y, Tian H, Li Y, Fan W. Lgr5 expression as stem cell marker in human gastric gland and its relatedness with other putative cancer stem cell

markers. Gene. 2013 Aug 1 and 2013, 525(1):18-25. doi: 10.1016/j.gene.2013.04.067. Epub. 2013 May 9. PMID: 23664892.

97. Zalzman M, Falco G, Sharova LV, Nishiyama A, Thomas M, Lee SL, Stagg CA, Hoang HG, Yang HT, Indig FE, Wersto RP, Ko MS. Zscan4 regulates telomere elongation and genomic stability in ES cells. Nature. 2010 Apr 8 and 10.1038/nature08882., 464(7290):858-63. doi: 10.1038/nature08882. Epub 2010 Mar 24. PMID: 20336070; PMCID: PMC2851843.

98. Ko SB, Azuma S, Yokoyama Y, Yamamoto A, Kyokane K, Niida S, Ishiguro H, Ko MS. Inflammation increases cells expressing ZSCAN4 and progenitor cell markers in the adult pancreas. Am J Physiol Gastrointest Liver Physiol. 2013 Jun 15 and 10, 304(12):G1103-16. doi: 10.1152/ajpgi.00299.2012. Epub 2013 Apr 18. PMID: 23599043; PMCID: PMC3680719.

99. Huang YH, Zhou XY, Wang HM, Xu H, Chen J, Lv NH. Aquaporin 5 promotes the proliferation and migration of human gastric carcinoma cells. Tumour Biol. 2013 Jun and 23436048., 34(3):1743-51. doi: 10.1007/s13277-013-0712-4. Epub 2013 Feb 23. PMID: 23436048.

100. Karasawa F, Shiota A, Goso Y, Kobayashi M, Sato Y, Masumoto J, Fujiwara M, Yokosawa S, Muraki T, Miyagawa S, Ueda M, Fukuda MN, Fukuda M, Ishihara K, Nakayama J. Essential role of gastric gland mucin in preventing gastric cancer in mice. J Clin Invest. 2012 Mar;122(3):923-34. doi: 10.1172/JCI59087. Epub 2012 Feb 6. PMID: 22307328; PMCID: PMC3287219.

101. Abdolahi F, Shahraki A, Sheervalilou R, Mortazavi SS. Identification of differentially expressed genes associated with the pathogenesis of gastric cancer by bioinformatics analysis. BMC Med Genomics. 2023 Dec 1 and 10.1186/s12920-023-01720-7., 16(1):311. doi: 10.1186/s12920-023-01720-7. PMID: 38041130; PMCID: PMC10690994.

102. Han S, Fink J, Jörg DJ, Lee E, Yum MK, Chatzeli L, Merker SR, Josserand M, Trendafilova T, Andersson-Rolf A, Dabrowska C, Kim H, Naumann R, Lee JH, Sasaki N, Mort RL, Basak O, Clevers H, Stange DE, Philpott A, Kim JK, Simons BD, Koo BK. Defining the Identity and Dynamics of Adult Gastric Isthmus Stem Cells. Cell Stem Cell. 2019 Sep 5;25(3):342-356.e7. doi: 10.1016/j.stem.2019.07.008. Epub 2019 Aug 15. PMID: 31422913; PMCID: PMC6739486.

103. Huh WJ, Esen E, Geahlen JH, Bredemeyer AJ, Lee AH, Shi G, Konieczny SF, Glimcher LH, Mills JC. XBP1 controls maturation of gastric zymogenic cells by induction of MIST1 and expansion of the rough endoplasmic reticulum. Gastroenterology. 2010 Dec;139(6):2038-49. doi: 10.1053/j.gastro.2010.08.050. Epub 2010 Oct 14. PMID: 20816838; PMCID: PMC2997137.

104. Hirao K, Natsuka Y, Tamura T, Wada I, Morito D, Natsuka S, Romero P, Sleno B, Tremblay LO, Herscovics A, Nagata K, Hosokawa N. EDEM3, a soluble EDEM homolog, enhances glycoprotein endoplasmic reticulum-associated degradation and mannose trimming. J Biol Chem. 2006 Apr 7;281(14):9650-8. doi: 10.1074/jbc.M512191200. Epub 2006 Jan 23. PMID: 16431915.

105. Hayano T, Sogawa K, Ichihara Y, Fujii-Kuriyama Y, Takahashi K. Primary structure of human pepsinogen C gene. J Biol Chem. 1988 Jan 25;263(3):1382-5. Erratum in: J Biol Chem 1988 Oct 5;263(28):14592. PMID: 3335549.