SUPPLEMENTAL DATA: Asem et al., ***Host Wnt5a Potentiates Microenvironmental Regulation of Ovarian Cancer Metastasis***

SUPPLEMENTAL METHODS.

Tumor Microarray. A tissue microarray (TMA) was constructed containing 51 primary serous ovarian carcinomas obtained from patients diagnosed with stage III and stage IV papillary serous ovarian carcinoma from The Medical Foundation (South Bend, IN) surgical pathology archives and arrayed as 0.5 mm diameter cores. Protocols were approved by the University of Notre Dame Institutional Review Board (IRB) and local hospital IRBs. TMA sections were stained with anti-Fgr or anti-phospho-Fgr (1:200) followed by a peroxidase-conjugated anti-rabbit-IgG and peroxidase detection using DAB chromogen. Slides were scanned into the eSlide Manager database (version 12.3.2.5030) with the Aperio ScanScope CS (Leica, Biosystems, Inc.). Stained tissues were scored by a board-certified pathologist [W.K.]. Each tissue was given a score up to +3, with 0 corresponding to negative staining, +1 to weakly positive staining, +2 to positive staining, and +3 to intense positive staining.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1: ***WNT5A*** **mRNA and Wnt5a protein expression in OvCa cell lines.**

(A) Relative expression of *WNT5A* was measured in OVCAR3, OVCAR5, OVCAR8, OVCA429, OVCA433 and DOV13 cell lines by quantitative PCR with reverse transcription. $n = 3$ independent biological replicates per experiments. One representative is shown from three independent experiment. **(B)** Wnt5a protein level was measured in conditioned media collected from duplicates of OVCAR3, OVCAR5, OVCAR8, OVCA429,

OVCA433 and DOV13, using ELISA assay. **(C)** Relative expression of Wnt5a-binding receptors; *ROR1, ROR2, FZD2, FZD4, FZD5* and *FZD7* was measured in OVCAR3, OVCAR5 and OVCAR8 cell lines by quantitative PCR with reverse transcription. $n = 3$ independent biological replicates per experiment. All results are presented as mean ± s.e.m. and statistical significance was calculated using a Student's two-tailed t-test. $P < 0.05$ is statistically significant.

Supplemental Fig. 2: Silencing of *WNT5A* in LP9 human peritoneal mesothelial cells.
(A) LP9 cells were transduced with eight different GFP-shRNA lentivirus particles targeting *WNT5A*. GFP images and merged images of GFP and phase contrast to show transduction efficiency in LP9 cells 10 days after transduction. **(B)** Wnt5a protein level was measured in conditioned media from the eight transduced LP9 cell lines, using ELISA assay. **(C)** Relative expression of *WNT5A* was measured in LP9 cells transduced with scrambled control lentivirus particles and a top candidate transduced LP9 cell line by quantitative PCR with reverse transcription. $n = 3$ independent biological replicates per experiment. One representative is shown from three independent experiments. All results are presented as mean ± s.e.m. and statistical significance was calculated using a Student's two-tailed t-test. $P < 0.05$ is statistically significant. **(D)** Adhesion to meso-mimetic cultures. RFP-tagged OVCAR5 and OVCAR8 cells were serum-starved overnight, treated with conditioned medium from control human peritoneal MC (LP9-CND) or from LP9 cells in which *WNT5A* is silenced (LP9-Wnt5a^{KD}-CND) for 24hr, then allowed to adhere to a meso-mimetic culture 20min and 1hr, respectively. Images of adherent cells obtained using Echo Revolve fluorescent microscope at 20x magnification. **(E)** Adhesion to murine peritoneal explants. RFP- tagged OVCAR5 and OVCAR8 cells were treated with either LP9-CND or LP9-Wnt5a^{KD}-CND for 24hr prior to incubation with murine peritoneal explants in

an *ex vivo* adhesion assay for 30min and 90min, respectively. Images of adherent cells obtained using Echo Revolve fluorescent microscope at 20x magnification. **(F)** Analysis of migration. OVCAR5 and OVCAR8 cells were serum-starved overnight, then cells were added to trans-well migration chambers containing LP9-CND or LP9-Wnt5a^{KD}-CND and incubated for 12hr and 18hr, respectively. Migrated cells were fixed and stained with Diff-Quik kit. Images of migrated cells obtained using Olympus BH-2 microscope. Scale bar = 20μm. **(G)** Analysis of invasion. OVCAR5 and OVCAR8 cells were serum-starved overnight, then cells were added to trans-well invasion chambers containing type I collagen gels (100 μl of 200ug/ml) with LP9-CND or LP9-Wnt5a^{KD}-CND and incubated for 24hr. Invaded cells were fixed and stained with Diff-Quik kit**.** Images of invaded cells obtained using Olympus BH-2 microscope. Scale bar = 50μm. (**H)** Adhesion to meso-mimetic cultures. RFP-tagged OVCAR3 cells were serum-starved overnight, then incubated with a Wnt5a neutralizing antibody (2 µg/ml) or control IgG for 24hr, then allowed to adhere to a meso-mimetic for culture 30 min. Images of adherent cells obtained using Echo Revolve fluorescent microscope at 20x. All experiments were done in triplicate with three independent biological replicates per each cell line. All results are presented as mean ± s.e.m. and *P*-values were calculated using a Student's two-tailed *t*-test. $P < 0.05$ is statistically significant.

Supplemental Fig. 3: *WNT5A* expression in murine tissues and longitudinal imaging of tumor burden in *WNT5A*-modified cohorts.

(A) Relative expression of *WNT5A* was measured in organs isolated from Wnt5a$^{fl/fl}$,UBC$^{Cre+/-}$,Vehicle control and Wnt5a^{KO},TMX by quantitative PCR with reverse transcription. Three mice per cohort were used. $n = 3$ independent biological replicates per experiment. All results are presented as mean ± s.e.m. **(B)** Murine cohorts were injected i.p. with 5x10^{6} RFP-tagged ID8-

Trp53$^{-/-}$ syngeneic murine OvCa cells. Beginning at 3 weeks post-injection, tumor burden *in situ* was longitudinally imaged weekly using a Bruker Xtreme In Vivo Imaging system. **(C)** Peritoneal lavage obtained from tumor-free mice with conditional WNT5A knockout or controls was analyzed using the murine Proteome Profiler Cytokine Array according to the manufacturer's specifications.

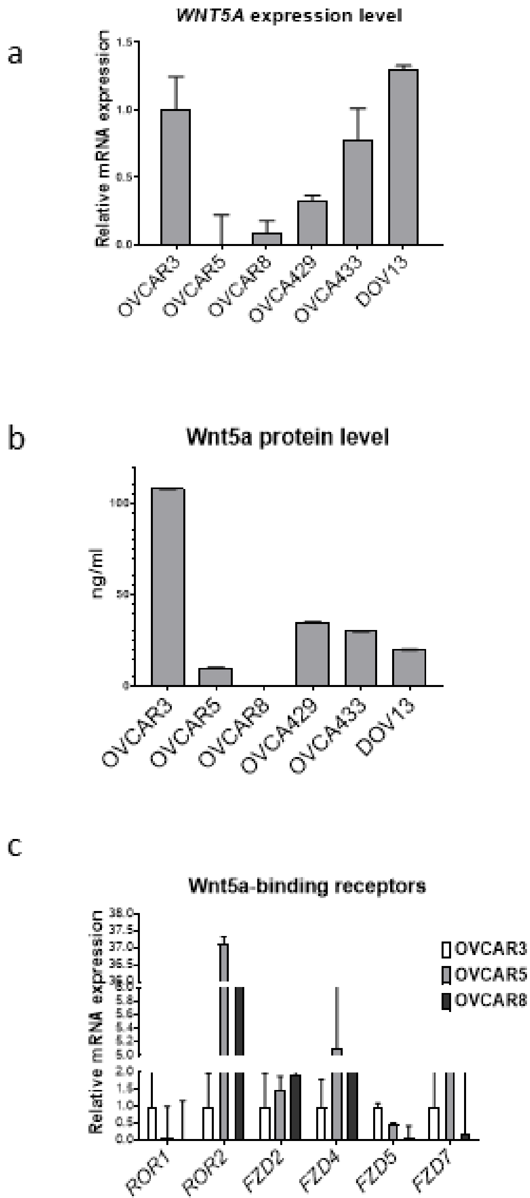
Supplemental Fig. 4: Fgr inhibition abrogates Wnt5a pro-metastatic cellular phenotypes.

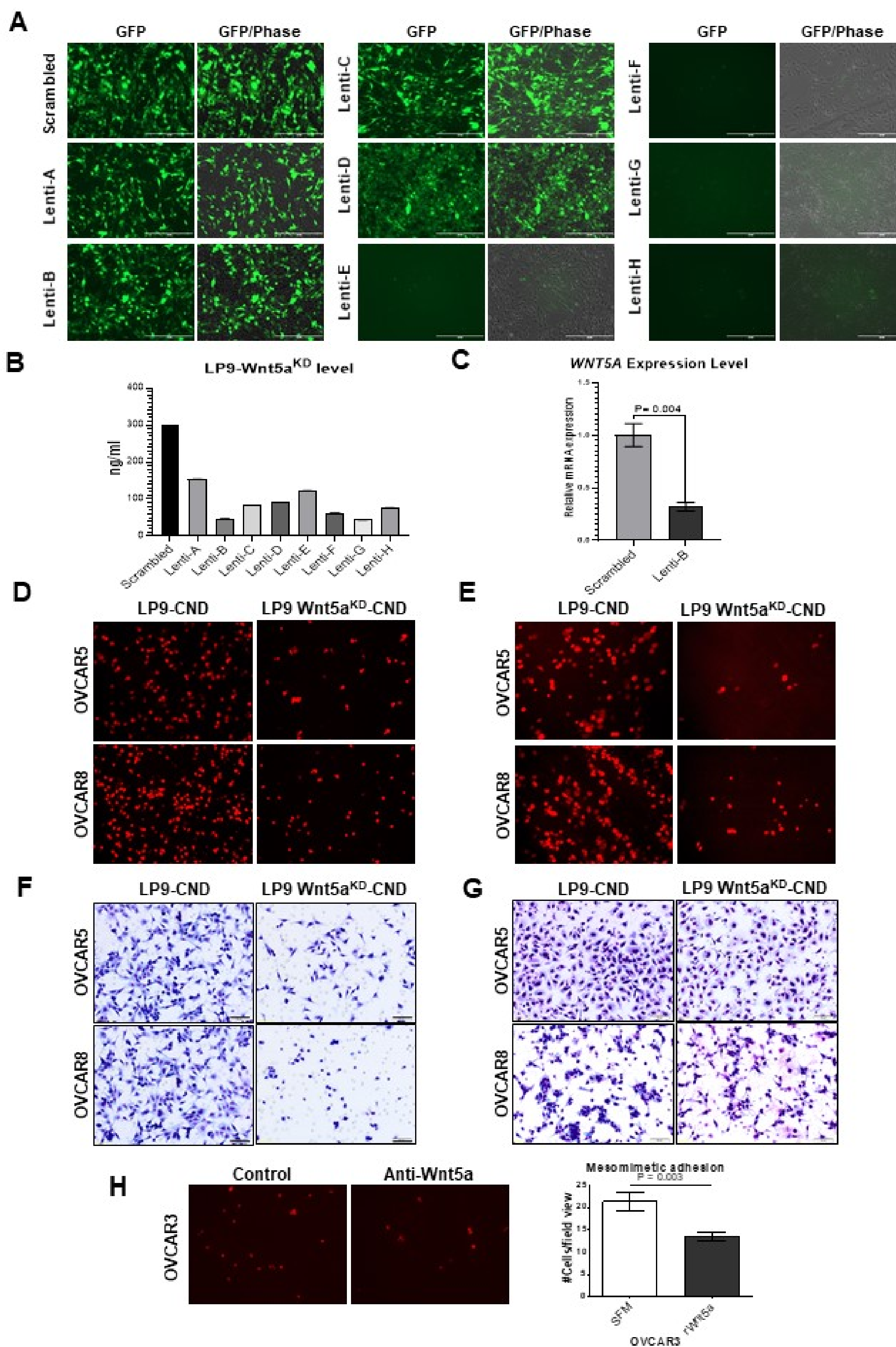
(A OVCAR5 and OVCAR8 cells were serum-starved overnight then incubated in SFM, or TL02-59 (0.01 µM or 1µM) for 6hr prior to addition of rWnt5a protein (0.4 µg/ml) for the time points indicated. Lysates were electrophoresed on SDS-polyacrylamide gels and immunoblotted with the antibodies noted. **(B,C)** RFP-tagged OVCAR5 and OVCAR8 cells were treated with SFM, rWnt5a (0.4 µg/ml), rWnt5a (0.4 µg/ml) and TL02-59 (1µM) or TL02-59 (1µM) for 24h as indicated prior to incubation with **(B)** mesomimetic culture for 20min or 1h, respectively, or with **(C)** murine peritoneal explants in an *ex vivo* adhesion assay for 30min or 90min, respectively. Images of adherent cells obtained using Echo Revolve fluorescent microscope at 20x magnification. **(D),** OVCAR5 and OVCAR8 cells were added to trans-well migration chambers containing SFM, rWnt5a (0.4 µg/ml), rWnt5a (0.4 µg/ml) and TL02-59 (1µM), or TL02-59 (1µM) and incubated for 12h and 18h, respectively. Migrated cells were fixed and stained with Diff-Quik kit. Images of migrated and invaded cells were obtained using Olympus BH-42 microscope. Scale bar = 50µm.

Supplemental Fig. 5. Fgr and phospho-Fgr protein levels in human serous ovarian carcinoma.

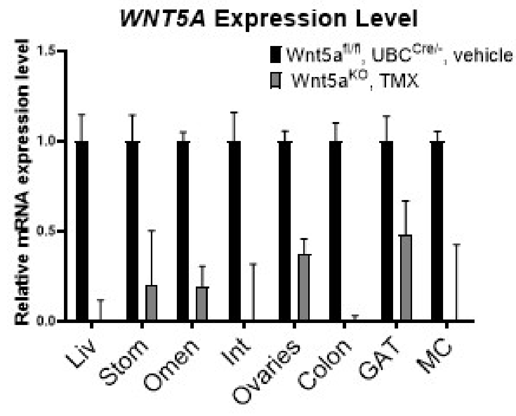
Representative images of Fgr and pFgr (Y412) immunohistochemical staining in a human serous papillary ovarian carcinoma tissue microarray containing stage III and stage IV tumors (n=51 samples). Tissues were incubated with anti-Fgr or anti-pFgr (1:200 dilution) followed by a peroxidase-conjugated anti-rabbit-IgG and peroxidase detection using DAB as described in Methods. Images were acquired with Olympus BH-42 microscope. Scoring for Fgr: 0, 10 (20%); 1+, 21 (41%); 2+, 13 (25%); 3+, 7 (14%). Scoring for phospho-Fgr: 0, 19 (37%); 1+ 21 (41%); 2+, 8 (16%); 3+ 2 (6%). Examples of tissues scored as 0, 1+, 2+, and 3+ are shown. Scale bar = 20 μm.

Asem et al., Suppl. Fig. 1





Asem et al., Suppl. Fig. 2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WNT5A Expression Level
Relative mRNA expression level
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Wnt5a KO, TMX
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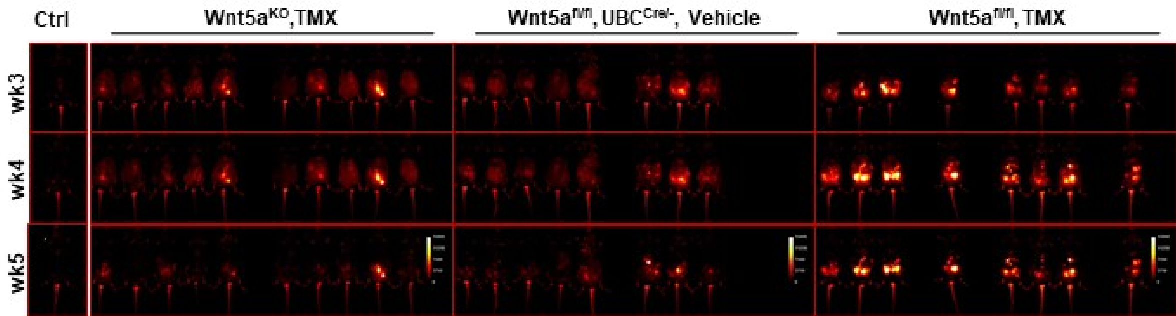
1.0
0.5
0.0
Liv
Stom
Omen
Int
Ovaries
Colon
GAT
MC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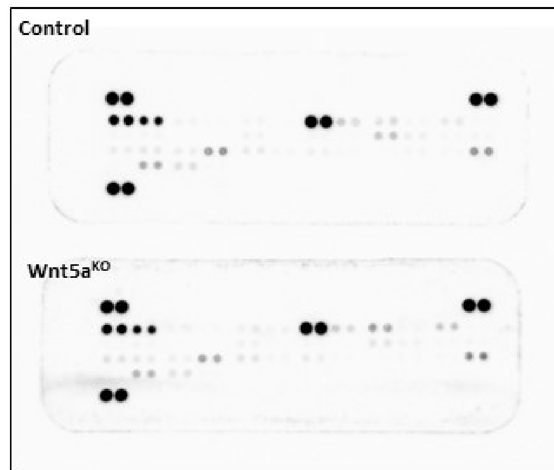
Wnt5a fl/fl, TMX
wk3
wk4
wk5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Control
Wnt5a KO

Asem et al., Suppl. Fig. 4

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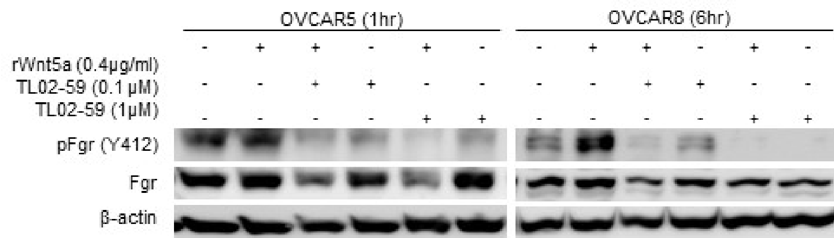

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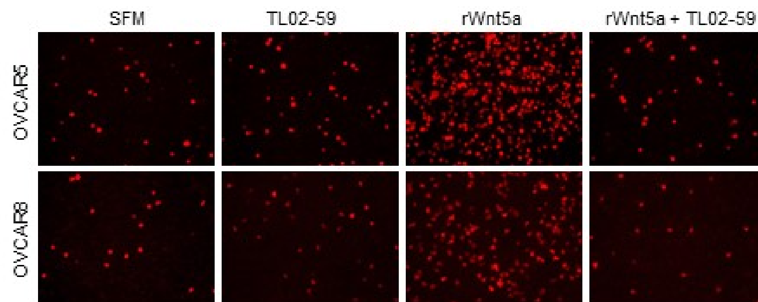

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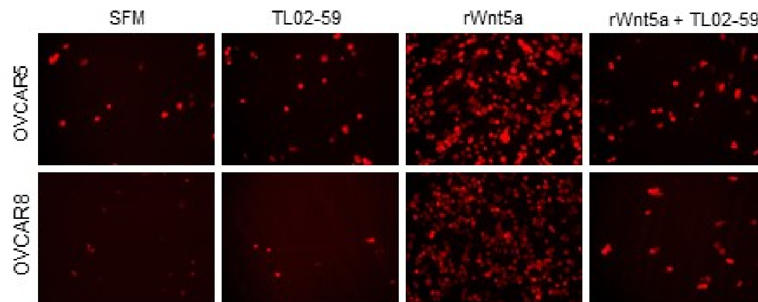

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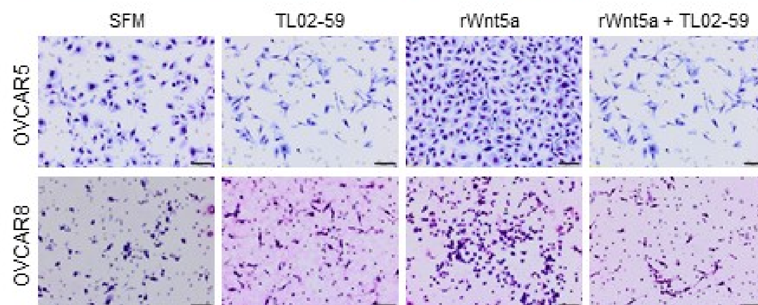
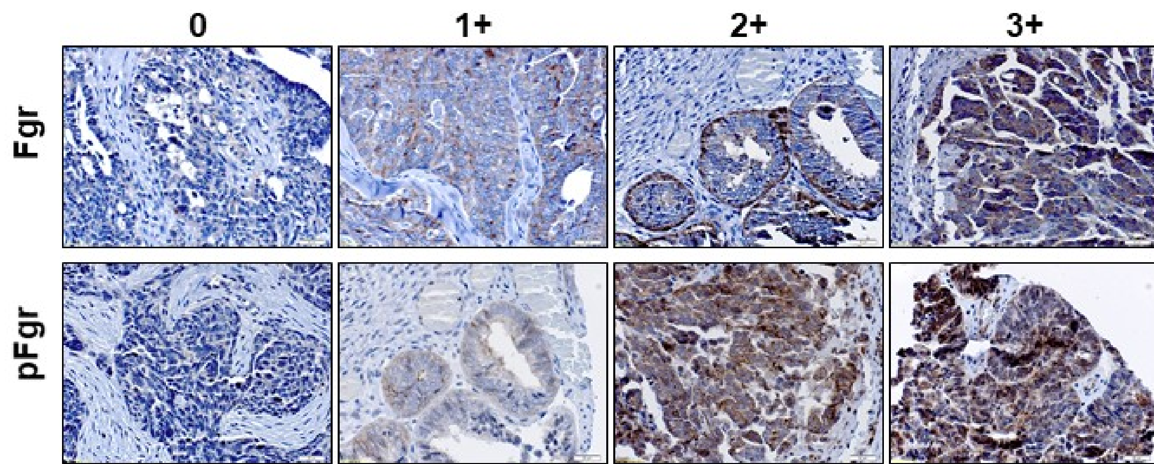


Asem et al., Suppl. Fig. 5



Supplemental Table. 1: Wnt5a shRNA lentivirus particles sequences.

Lenti-A	GAAGAAACTGTGCCACTTGTATCAGGACC
Lenti-B	CCACTCATGCTTCTCCTATTGTACTGCAG
Lenti-C	AAGGTAATTGCGTGCCATTCAGCACTGCA
Lenti-D	CGAAAGGCTTATGCCAAATGGAAGATAGA
Lenti-E	AACTGTGCCACTTGTATCAGGACCACATG
Lenti-F	CAATTCCGACATCGAAGGTGGAACTGCAG
Lenti-G	CGTGGCTACGACCAGTTCAAGACCGTGCA
Lenti-H	TCCTGTTACCATCTAAGAACTCTGTGGTT

Supplemental Table. 2: Primers for qPCR

		Forward	**Reverse**
mouse	Wnt5a	GGTGAGGGACTGGAAGTTGC	GGAGCAGATGTTTATTGCCTT
	RPS13	CCCAGGTCCGTTTTGTGACT	GTGCTTTCGGACAGCAACAG
	UBC-Cre	GACGTCACCCGTTCTGTTG	AGGCAAATTTTGGTGTACGG
Human	wnt5a	ATTCTTGGTGGTCGCTAGGTA	CGCCTTCTCCGATGTACTGC
	RPS13	CGAAGGCATCTTGAGAGGAACA	TCGAGCCAAACGGTGAATC
	Ror1	ACCGCACCGTGTATATGGAGTCT	GCATAGTGGCACAGGGAAGG
	Ror2	GGCAGAACCCATCCTCGTG	GGACTGCGAATCCAGGACC
	Fzd2	GAAAGGCTGGAGCGGCTCAT	TGGTGAGGCGAGTGTAGAACT
	Fzd5	CTTGTTTCCAAAGTCCAATCAAGT	GCCTACTCTTCACCCTTCTTTAAC G
	Fzd7	GCCTCGTCGCACTCCTCAG	GGGGCTCATACCGCAGTCTC
	Fzd4	GCCAATGTCCACAGAGAAGA	AGGCAAACCCAAATTCTCTCA