Supplemental Material Table of Contents

Supplemental Table 1. RNA sequencing identification of proximal tubule marker expression in HPTC

Ensembl gene ID	Gene name	CPM	Expression Ranking
ENSG00000245532	NEAT1	426.052957	298
ENSG00000054654	SYNE2	140.832669	1355
ENSG00000099250	NRP1	112.058187	1796
ENSG00000090565	RAB11FIP3	66.0499293	>2000
ENSG00000022556	NLRP2	43.4322078	>2000
ENSG00000111961	SASH1	34.4933133	>2000
ENSG00000048471	SNX29	17.774747	>2000
ENSG00000069020	MAST4	14.1940371	>2000
ENSG00000066230	SLC9A3	13.3954615	>2000
ENSG00000173068	BNC2	12.5711254	>2000
ENSG00000169760	NLGN1	2.29268475	>2000
ENSG00000262619	LINC00621	0.9016176	>2000
ENSG00000158296	SLC13A3	0.85009659	>2000
ENSG00000100031	GGT1	0.85009659	>2000
ENSG00000153707	PTPRD	0.20608402	>2000
ENSG00000107611	CUBN	0.20608402	>2000
ENSG00000166415	WDR72	0.05152101	>2000
ENSG00000228262	LINC01320	0.0257605	>2000

CPM: Counts per million

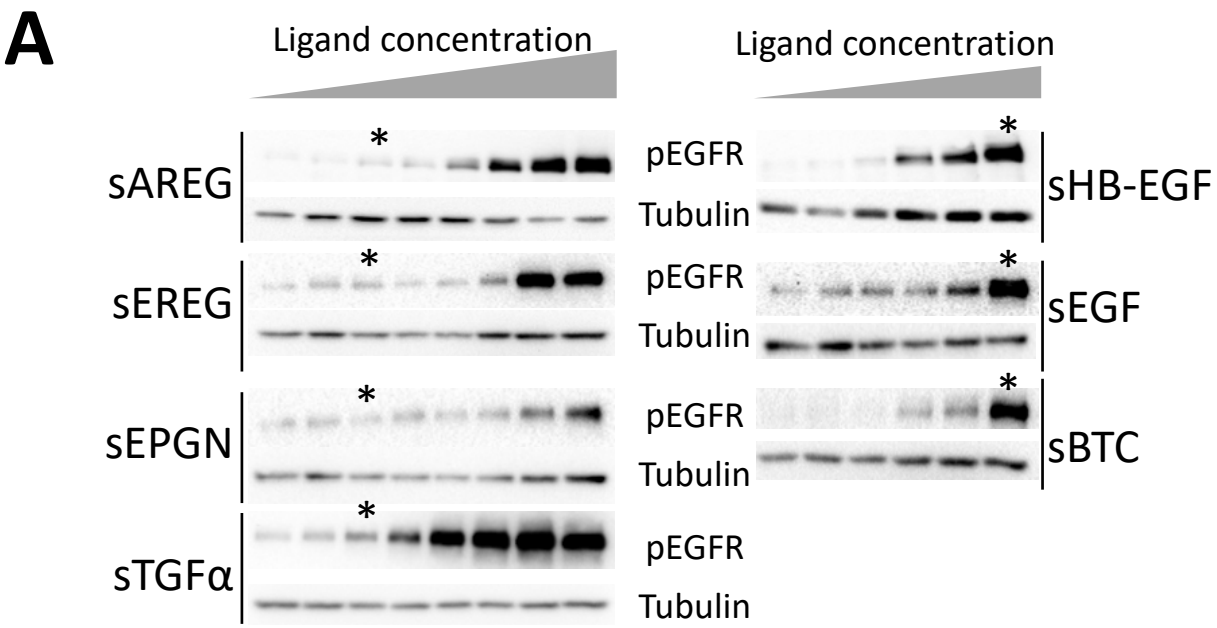
Supplemental Table 2. qPCR primers list

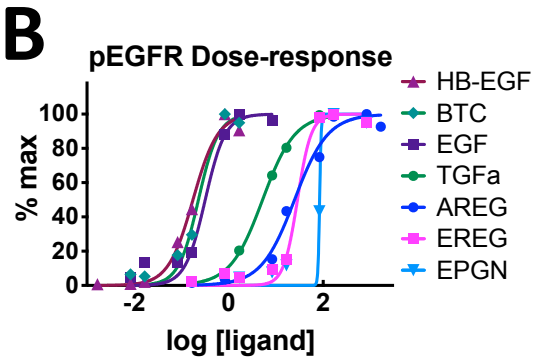
Target gene	Synonym	Species	Direction	Sequence
ADAM17	TACE	human	Forward	AGTGCAGTGACAGGAACAGT
ADAM17	TACE	human	Reverse	GGACACGCCTTTGCAAGTAG
AREG		human	Forward	TCGGCTCAGGCCATTATGC
AREG		human	Reverse	AATCCATCAGCACTGTGGTCC
EGF		human	Forward	CTTGGGAGCCTGAGCAGAAA
EGF		human	Reverse	TGCACAAGTGTGACTGGAGG
EGFR		human	Forward	CGAATGGGCCTAAGATCCCG
EGFR		human	Reverse	CTTCGCATGAAGAGGCCGAT
EPGN		human	Forward	ACAGAAGCTGACAACATAGA
EPGN		human	Reverse	AGCTCATGGTGGAATGCACA
EREG		human	Forward	ACGTGTGGCTCAAGTGTCAA
EREG		human	Reverse	CACTTCACACCTGCAGTAGTT
GAPDH		human/mouse	Forward	ACCACAGTCCATGCCATCAC
GAPDH		human/mouse	Reverse	TCCACCACCCTGTTGCTGTA
HBEGF		human	Forward	TTGTGCTCAAGGAATCGGCT
HBEGF		human	Reverse	CAACTGGGGACGAAGGAGTC
PRLP0		human	Forward	CGTCCTCGTGGAAGTGACAT
PRLP0		human	Reverse	TAGTTGGACTTCCAGGTCGC
TGFA		human	Forward	GTAAAATGGTCCCCTCGGCT
TGFA		human	Reverse	GGGTCTGCACTCAGCGG
YAP1	YAP	human	Forward	TGATGGATGGGAACAAGCCA
YAP1	YAP	human	Reverse	TGGTTCATGGCAAAACGAGG
Acta2	αSMA	mouse	Forward	AGCCATCTTTCATTGGGATGC
Acta2	αSMA	mouse	Reverse	TACCCCCTGACAGGACGTTG
Adam17	Tace	mouse	Forward	TCTGAAGAGTTTGTTCGTCGA
Adam17	Tace	mouse	Reverse	CTTCTCCACGGCCCATGTAT
Areg		mouse	Forward	GCTGAGGACAATGCAGGGTA
Areg		mouse	Reverse	GTGACAACTGGGCATCTGGA
Ccl2	Mcp1	mouse	Forward	CACTCACCTGCTGCTACTCA
Ccl2	Mcp1	mouse	Reverse	GCTTGGTGACAAAAACTACAC
Ccl3	Mip1A	mouse	Forward	CAGCCAGGTGTCATTTTCCTG
Ccl3	Mip1A	mouse	Reverse	TCTCAGGCATTCAGTTCCAGG
Ccl5	Rantes	mouse	Forward	CTCACCATATGGCTCGGACA
Ccl5	Rantes	mouse	Reverse	CGACTGCAAGATTGGAGCAC
Egf		mouse	Forward	AGCATACTCAGCGTCACAGC
Egf		mouse	Reverse	GCAGGACCGGCACAAGTC
Egfr		mouse	Forward	ACCTCTCCCGGTCAGAGATG
Egfr		mouse	Reverse	CTTGTGCCTTGGCAGACTTTC
Epgn		mouse	Forward	AACAACACCGAAGCTGACTAC

Supplemental Table 2 (cont.)

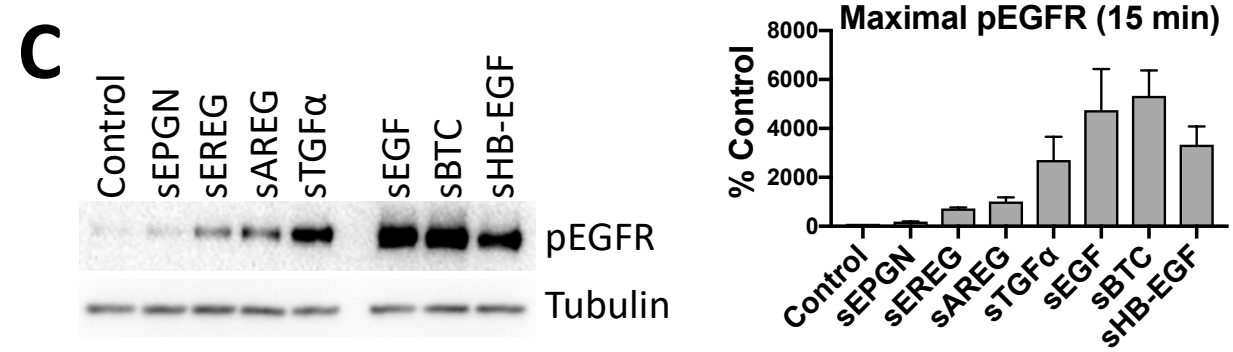
Epgn		**mouse**	**Reverse**	**TGGTGGAATGCACATGCTCC**
Ereg		mouse	Forward	TGCTTTGTCTAGGTTCCCACC
Ereg		mouse	Reverse	GGCGGTACAGTTATCCTCGG
Hbegf		mouse	Forward	TCTGGCCGCAGTGTTGTCC
Hbegf		mouse	Reverse	GGTTTGTGGATCCAGTGGGA
Prpl0		mouse	Forward	CTCTCGCTTTCTGGAGGGTG
Prpl0		mouse	Reverse	ACGCGCTTGTACCCATTGAT
Tgfa		mouse	Forward	CTCTGCTAGCGCTGGGTATC
Tgfa		mouse	Reverse	TGGGCACTTGTTGAAGTGAG
Tgfb1	Tgfβ	mouse	Forward	CTGCTGACCCCCACTGATAC
Tgfb1	Tgfβ	mouse	Reverse	AGCCCTGTATTCCGTCTCCT
Tnf	Tnfa	mouse	Forward	ATGGCCTCCCTCTCATCAGT
Tnf	Tnfa	mouse	Reverse	CTTGGTGGTTTGCTACGACG
Yap1	Yap	mouse	Forward	TTCGGCAGGCAATACGGAAT
Yap1	Yap	mouse	Reverse	CATCCTGCTCCAGTGTAGGC

Suppl. Fig. 1: Dose-response curves and maximal EGFR phosphorylation by its different ligands in HPTC.



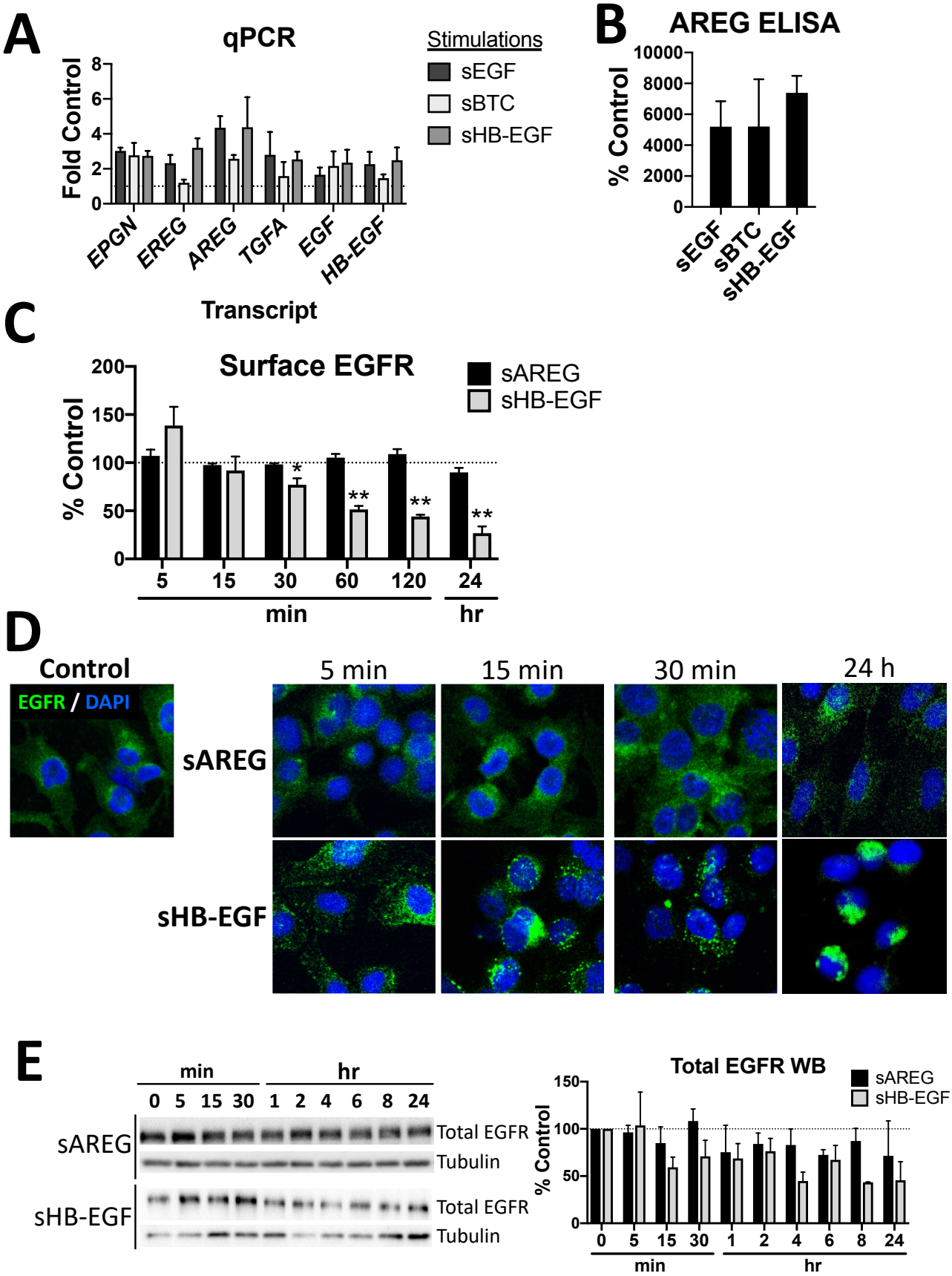


	sHBEGF	sBTC	sEGF	sTGFα	sAREG	sEREG	sEPGN
EC50 (nM)	0.18	0.23	0.32	5.23	24.81	28.6	~84

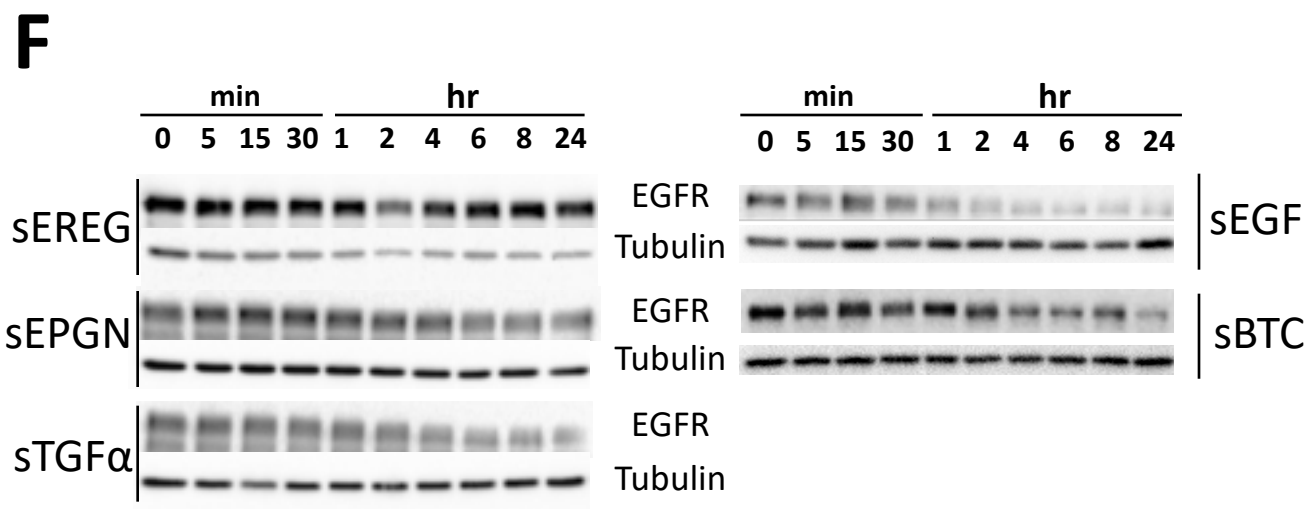


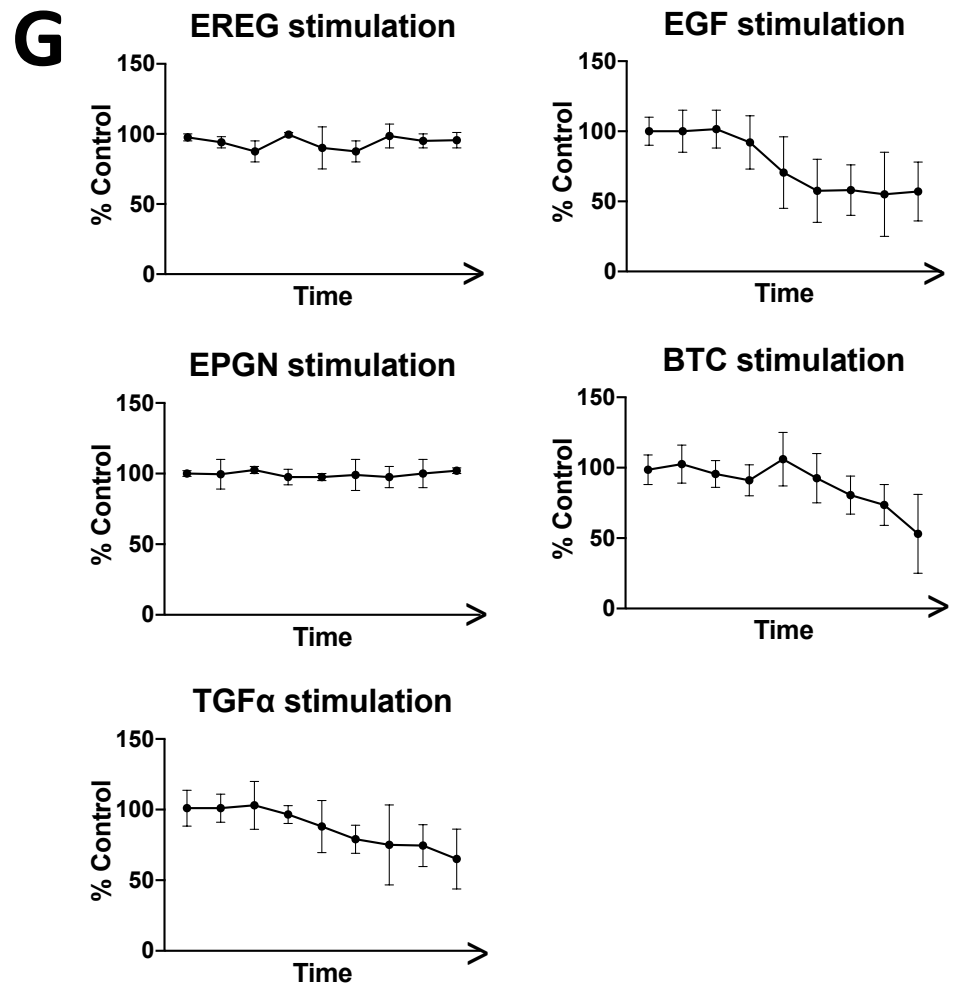
Supplemental Figure 1: EGFR ligand dose response in HPTC. (A-B) Serum starved HPTCs were treated for 15 min with increasing amounts of EGFR ligands and EGFR phosphorylation was tested by WB (A). A concentration range of 85 fM – 8.5 pM was used for high affinity and of 1.7 pM – 17 nM for low affinity ligands (stars denote concentration of 8.5 pM in all panels). Dose-response curves (B, right graph) and EC50 values (B, table) were calculated after normalization to 100% of maximal phosphorylation obtained by each ligand. **(C)** HPTCs were treated for 15 min with EGFR ligands in amounts sufficient to induce maximal EGFR phosphorylation by each ligand. EGFR phosphorylation was examined by WB (C, left panel) and densitometric analysis (C, column graph) was performed after normalization to non-stimulated cells. Tubulin was used in all experiments for normalization (loading control).

Suppl. Fig. 2: High affinity ligands also induce AREG release but they lead to internalization of EGFR.



Suppl. Fig. 2 (cont.)





Supplemental Figure 2. sAREG and sHB-EGF differentially regulate EGFR cell-surface levels in HPTCs. (A) HPTCs were treated with different EGFR ligands (shown in the legend) for 24h and mRNA expression of endogenous EGFR ligands (shown in x-axis) was tested by qPCR. Results are presented after normalization to control-stimulated cells (fold control). **(B)** Endogenous sAREG released in HPTC culture medium was measured by ELISA after stimulation with soluble high affinity EGFR ligands. Results are presented as percentile of sAREG concentration in medium of control-stimulated cells (% Control). **(C)** HPTCs were treated with equimolar amounts of sAREG or sHB-EGF and surface levels of EGFR were measured by flow cytometry at different time points as noted. Results are presented after normalization to surface EGFR of control-stimulated cells corresponding to each respective time point (% Control). **(D)** HPTCs were treated with equimolar amounts of sAREG or sHB-EGF and EGFR cellular distribution was examined by immunocytochemistry at different time points as noted. **(E)** HPTCs were treated with equimolar amounts of sAREG or sHB-EGF and total EGFR levels were examined by western blot (left panels) and quantified by densitometric analysis (right panel) at different time points as noted. Tubulin was used as loading control. **(F, G)** HPTCs were treated with equimolar amounts of EGFR ligandsand total EGFR levels were examined by western blot (top panels) and quantified by densitometric analysis (bottom panels) at different time points as noted. Tubulin was used as loading control. n=3-4 for all experiments, *: $P<0.05$, **: $P<0.01$.