

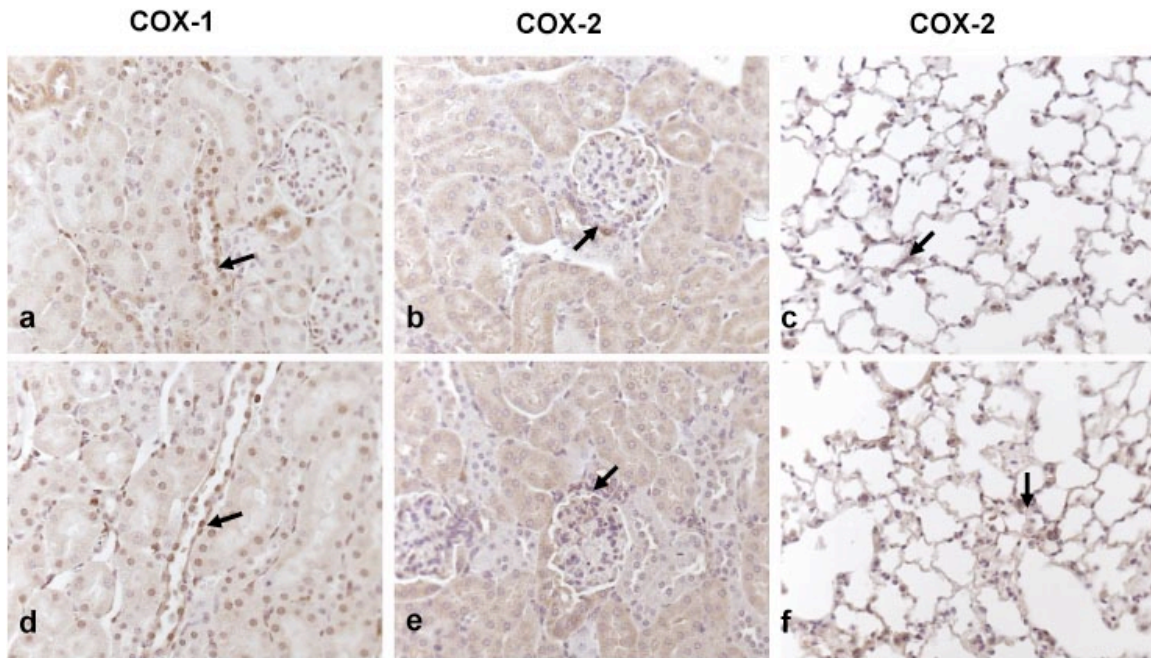
SUPPLEMENTAL MATERIAL

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Supplemental Data
Figure 1S



Microscopy through a partially closed condenser diaphragm showed the PGT-positive cells (brown reaction product, red arrow) to have the birefringent lamellar bodies (black arrow) characteristic of type II alveolar epithelial cells²⁰.

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Supplemental Data Figure 2S
COX-1 and COX-2 immunohistochemistry in rescued adult PGT^{-/-} mice



a,b,c: PGT^{+/+} kidney (a, b) and lung (c);
d,e,f: PGT^{-/-} (d, e) and lung (f).

COX-1 was expressed in cortical collecting duct (a,d, arrows); COX-2 was expressed in macula densa (b, e arrow) and macrophages in lungs (c, f arrows).

There was no significant difference in COX-1 or COX-2 staining intensity between PGT^{-/-} and ^{+/+} mice.

Lungs and kidneys of 5-7 month old PGT^{-/-} (n = 4) and PGT^{+/+} (n = 5) mice were examined. Paraffin sections were subjected to immunohistochemical analysis using standard methods as above. Sections were incubated overnight at 4°C with polyclonal rabbit anti-mouse COX-1 (1:200 dilution; Cat.# 160109; Cayman Chemical, Ann Arbor, MI) and anti-mouse COX-2 antisera (1:200 for kidney, 1:400 for lung; Cat.# 160126; Cayman Chemical). Sections were then incubated with biotin-conjugated goat anti-rabbit IgG for 1 hour at room temperature. Visualization was by DAB as described above.

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Table 1S. Primer Pairs Used for PCR to Detect of all Phenotypes

Primer Site	Primers	Amplimer Size (kb)*
Outside of Neo gene (1)	5'-GTTCTTCGGACGCCTCGTCAACAC-3'	2.5
	5'-ACTCATCTATGACCATAACCACCCTG-3'	
Inside of Neo gene (2)	5'-AGGTGAGATGACAGGAGATC-3'	1.1
	5'-TGCCTGTGCTCTGACTTGCATCTC-3'	
Wild type AA' (3)	5'-TGTCAGTTCTCTCCCTCACATCG-3'	2.8
	5'-TGGCTAAACTCCTCTGCATCCC-3'	
Wild type BB' (4)	5'-ATACGCGGTCTCCTCTTCCT-3'	1.0
	5'-CAAGGTGCTGGCTAAACTCC-3'	
Third LoxP (5)	5'-CAAGCAGGAGGACCTGAGTT-3'	0.18
	5'-CCCCACGTGTTTCTCAGATG-3'	0.21

*kb: kilo basepair

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 Table 2S.

Real-time PCR on mouse embryos and on lung, hear, and kidney of adult rescued PGT -/- mice (n = 5) compared to adult PGT +/+ mice (n = 6).

P values were obtained by t-test.

Bold values for P are statistically significant.

Mouse embryo

Gene	mPGT	Cox1	Cox2	EP2	EP4
PGT -/-	0.115	0.976	0.897	0.959	0.716
SEM	0.017	0.118	0.119	0.123	0.083
PGT +/+	1.206	1.048	1.067	0.953	0.933
SEM	0.104	0.032	0.047	0.052	0.100
P=	0.008	0.650	0.366	0.970	0.196

Mouse lung

Gene	mPGT	Cox1	Cox2	EP2	EP4
PGT -/-	0.010	0.556	0.237	0.500	0.349
SEM	0.002	0.105	0.053	0.121	0.067
PGT +/+	1.442	1.125	0.575	0.893	0.605
SEM	0.366	0.181	0.143	0.151	0.136
P=	0.030	0.043	0.095	0.091	0.161

Mouse heart

Gene	mPGT	Cox1	Cox2	EP2	EP4
PGT -/-	0.138	0.884	1.214	1.889	1.362
SEM	0.032	0.107	0.340	0.208	0.208
PGT +/+	0.678	0.734	0.721	1.035	0.771
SEM	0.116	0.091	0.174	0.118	0.161
P=	0.014	0.186	0.211	0.012	0.053

Mouse kidney

Gene	mPGT	Cox1	Cox2	EP2	EP4
PGT -/-	0.155	1.595	1.684	2.203	3.289
SEM	0.032	0.164	0.174	0.182	0.249
PGT +/+	1.128	1.249	2.030	1.787	1.617
SEM	0.233	0.159	0.397	0.346	0.154
P=	0.024	0.267	0.271	0.560	0.002