Supplementary Figure 1. **Analysis of the sequence and predicted domain structure of human *SCCRO*. *a.*** Partial cDNA sequence containing the entire open reading frame and the deduced amino acid sequence of human *SCCRO*. Translation initiation sequence and the polyadenylation signal are underlined. ***b.*** Predicted domain structure of *SCCRO* showing the three putative functional domains. ***c.*** Northern blot analysis of *SCCRO* mRNA in various human tissues using a *SCCRO* cDNA probe with the highest expression seen in the heart and skeletal muscle.

Supplementary Figure 2. **Protein alignment of *SCCRO***. A multiple sequence alignment of proteins that are identical/similar to *SCCRO* was constructed by parsing BLAST-generated high scoring pairs of sequence and realigning them with CLUSTAL W, followed by manual annotation. Residues that are conserved across all the indicated species are marked with an asterisk.

Supplementary Figure 3. **Assessment of *SCCRO* antibody for Western blotting**. ***a.*** To confirm that the anti-*SCCRO* antibody specifically detects *SCCRO*, we ran whole cell extract from cancer cell line MDA1386 along with purified bacterially expressed *SCCRO*, GST-SCCRO and GST. Although *SCCRO* antibody detected other proteins, the prominent band in the cell extract co-migrated with the purified protein. ***b.*** The sensitivity of the antibody was assessed by loading a concentration gradient of bacterially expressed *SCCRO* protein. This showed that *SCCRO* protein levels as low as 62.5pg could be detected.

Supplementary Figure 4. **Assessment of *SCCRO* antibody for immunohistochemistry.** ***a, b*.** Results from immunohistochemistry using the anti-*SCCRO* antibody correlate well

with those from Western blot, showing strong expression in MDA1438 cells (*a*) but not in 584 cells (*b*). ***c.*** *SCCRO* expression was seen in primary lung cancer cells and was absent in the stroma. The staining was both cytoplasmic and nuclear (inset). ***d.*** Preincubation of *SCCRO* antibody with the peptide against which it was raised resulted in loss of staining, suggesting that the antibody specifically detected *SCCRO*. ***e, f.*** Absence of staining in histologically normal oral mucosa or lung.

Supplementary Figure 5. SCCRO **expression in various cell lines.** Western blot showing the relative levels of *SCCRO* in different cell lines. Expression is highest in cancer cell lines carrying amplification (SCC15 and MDA1386).

Supplementary Figure 6. SCCRO **expression in primary lung tumors.** Western blot showing *SCCRO* expression in primary lung tumors and histologically normal lung tissue. These samples correspond to the cases in which immunohistochemical expression analysis was done, as in Figure 3c.

Supplementary Figure 7. **SCCRO expression during embryogenesis.** We generated mice from ES cells with a viral insertional mutation containing lacZ insertion in the first intron of the *SCCRO* such that *SCCRO* exon 1- lacZ fusion is generated (Kaufman A, et al. Manuscript in preparation). ***a.*** X-gal staining of E9.5 mouse embryos carrying lacZ insertion in *SCCRO* revealed beta-galactosidase activity in the developing forebrain, midbrain, and hindbrain, with the strongest expression observed in the forebrain and midbrain. In addition, SCCRO expression was also detected in the 1st and 2nd branchial arches, developing myocardium and mesenchymal cell of the somites. ***b.*** At E10.5 staining becomes more pronounced in the mandibular and maxillary portions of the first

branchial arch and the developing limb buds. The observation that the temporal and spacial expression of *SCCRO* is similar to the reported expression of *Gli1* further suggests a relationship between the two genes. (http://dev.biologists.org/content/vol129/issue20/images/large/DEV4644F3.jpeg)