Supplemental Figure 1. Total Vegf protein levels in the developing brain. E11.5 mouse brains were microdissected, isolating the forebrain and midbrain regions, and total protein extracted. Vegf protein levels were quantified by ELISA detecting total levels in the forebrain and midbrain based on a VEGF protein standard curve. There was no statistical difference in total Vegf protein between the wild type and Vegf isoform mice in either the forebrain or midbrain. (N=7; 1-way ANOVA, forebrain P-value = 0.2442, midbrain P-value = 0.3591).

Supplemental Figure 2. Cross-comparisons of transcriptional shifts between Vegf isoform forebrains and embryonic time points. Zcuts from Vegf isoform mice versus E9.5 wild type were plotted on the X and were compared to Zcuts from E11.5 wild types versus E9.5 wild types on the Y axis. Scatter plots compare shifts in gene expression of the individual E9.5 Vegf isoforms versus E11.5 wild types all relative to an E9.5 wild type baseline. Genes are sorted based on being significant in any one group but not the other

(Blue and Green boxes), significant in both groups (Red boxes), or not significant in either (Grey boxes). Genes found in the upper-right and lower-left quadrants are shifted in the same direction relative to the E9.5 wild type base line.

As a control for our ComBat.R batch normalization, E9.5 wild type forebrains from our two technical batches (WT A, n= 4 (Darland et al., 2011), and WT B, n = 2) were compared to those from and Hartl et al., 2008 (WT C, n = 6) (Hartl et al., 2008) and plotted on a scatter plot comparing WT A versus WT B on the x-axis and WT A versus WT C on the y-axis. Zcuts were obtained from three separate wild type technical batches using a Bayesian-modeled run in BAMarray. No genes were detected as significantly changed in any of the different technical batches, demonstrating a proof of principle for our meta-analysis approach (D).

Supplemental Figure 3. Vegf regulation of NSC proliferation and differentiation. E11.5 wild types (A, E, I), Vegf120 (B, F, J), Vegf188 (C, G, K), and Vegf120/188 (D, H, L) forebrains were sectioned at 30 μm in the parasagittal plane and immunolabeled with pHH3 (A-D, I-L) or Tbr2 (E-H) and counter-stained with methyl-green. The whole forebrain imaged at 2x showed no overt structural differences between the Vegf isoform mice versus wild type (A-D, scalebar = 500 μm). At 40x magnification Tbr2-positive cells were labeled uniformly along the pial surface (E-H), and pHH3-positive cells lined the ventricular surface (I-L, scalebar = 50 μm). Sections were cut at 30 μm so that multiple optical planes could be counted using stereological methods; a single plane of focus is shown for each image.

Supplemental Figure 4. The ratio of cleaved Caspase 3 to uncleaved Caspase 3 is unchanged in the Vegf isoform mice at E11.5. As an assessment of apoptosis, western blots were used to quantify levels of cleaved and uncleaved Capase 3 in forebrain-derived lysates of E11.5 wild type and Vegf isoform mice (A). Band densities for Caspase 3, cleaved Caspase 3, alpha-Tubulin, and Elongation Factor 1-alpha (EF1-α) were quantified using ImageJ (NIH Image/Image J). Band densities for Caspase 3 and cleaved Caspase 3

were normalized against the loading control EF1-α, and the ratios of cleaved versus uncleaved Caspase 3 were obtained (B). A one-way ANOVA detected no statistical difference among any of the groups (N = 6, P-value = 0.7215, this experiment was repeated 3 times with six different embryos from 3 separate litters).

Supplemental Figure 5. Shifts in Vegf isoform profile alter neocortical layering at postnatal day 0 (P0). Coronal sections from P0 wild type (A, B), Vegf120 (C), Vegf188 (D), and Vegf120/188 (E) mice were stained with hematoxylin and eosin. A 20X magnification was used to image the most caudal sections of the lateral ventricle in the forebrain prior to the emergence of the hippocampus and dentate gyrus. A 2x objective picture of a wild type (A) is included to indicate position within the cutting plane (scale bar in A is 1000 μm; scale bar in B-E is 100 μm). The developing cortical layers are indicated, layer I (black bar), layers II-IV (blue bar), layer V (red bar), and layer VI (green bar). The distribution of cell nuclei in layers V and VI are shifted in the Vegf isoform mice relative to wild type.

Supplemental Figure 6. Shifts in Vegf isoform profile alter formation of neocortical layer V at P0. Layer V marker Ctip2 (red) and nuclear marker DAPI (blue) are shown at P0 in wild type (A), Vegf120 (B), Vegf188(C), and Vegf120/188 (D). The vertical region is located in the caudal-most sections of the lateral ventricles (areas equivalent to those shown in Supplemental Figure 5). Ctip2 was detected in layer V of all the mice with a more dorsal distribution in wider bands detected in the Vegf188 and Vegf120/188 mice (indicated by chevrons A-D, dashed yellow line A'-D'). Several Ctip2-positive cells were detected in layer VI in the wild type and Vegf120 mice (arrowheads). Ctip2 labeling was present throughout the caudate putamen (CP) in all animals. An isotype-matched negative control is shown for comparison (E). (scalebar is 125 μm in A-D; scalebar is 250 μm in A'-D').