**Supplementary Figure Legends**

**Supplementary Figure 1: *Spontaneous brain macrometastases analyzed by MRI or histology.*** T1-weighted MRI images from: A, Normal mice (n=3), and B-F, injected mice analyzed for brain macrometastases 3-6 months following resection of primary tumors (n=8). Scale bars=3mm. Arrow and dashed circles mark lesions. Gd=Gadolinium. G, Summary of lesion volumes analyzed. Red dots represent sum of volumes in mice that presented with two lesions. H-N, H&E, mCherry fluorescence or mCherry IHC images of macrometastases. In J, scale bar=250µm, all other scale bars=100µm. V=ventricle, CR=cerebellum, BP=brain parenchyma, Met=macrometastases.

**Supplementary Figure 2: *Molecular analysis of mCherry enables detection of micrometastases.*** A, Representative qPCR analysis of mCherry expression in local cutaneous tumors or in normal brain. Results were normalized to *Hprt*. Error bars represent RQ min and max, n=3. #=undetected expression. B, Calibration curve for quantification of lung metastases, n=3. Error bars represent SD of technical repeats, #=undetected expression. C, Metastatic load in a cohort of spontaneous lung metastases-bearing mice (n=7) determined utilizing qPCR for mCherry in reference to the calibration curve in B. D-G, mCherry fluorescence or mCherry IHC images of parenchymal micrometastases, arrows indicate lesions. All scale bars=100µm. H-K, mCherry fluorescence or mCherry IHC images of micrometastases in the choroid plexus, arrows indicate lesions, K includes inset. In K, scale bar=100µm, all other scale bars=50µm.

**Supplementary Figure 3: *FACS analysis enables the detection and quantification of brain micrometastases.*** A, Normal brains were admixed with known numbers of melanoma cells followed by combined digestion to single cell suspension. Samples were analyzed for the presence of viable mCherry cells. B, Normal brain and normal brains spiked with 102-104 RMS cells. C, Calibration curve of metastatic load analyzed by FACS. Y axis: fold change in number of detected mCherry+ cells per 10,000 events, normalized to control mice. Results are representative of three independent experiments.

**Supplementary Figure 4: *Detection and molecular quantification of CTCs.*** A-B, Calibration for detection of circulating tumor cells: qPCR of mCherry (A) or *Trp-2* (B) expression in peripheral blood spiked with melanoma cells. Representative results of two independent experiments. C, qPCR analysis of mCherry expression in blood samples from injected mice. Representative from 2 cohorts analyzed, n=14. Results are normalized to *Hprt*, and to the signal of 102 cells. Error bars represent RQ min and max of technical repeats. #=undetected expression. D, Correlation between positive blood samples and brain metastases, n=16 (Fisher’s exact test).

**Supplementary Figure 5**: ***Melanoma-derived transcripts can be found in CSF******isolated from intracardiac injected* *mice.*** A-B, qPCR analyses of CSF from injected mice (n=2) or control mice (n=2) for: A, *Mart-1* and B, mCherry. La=ladder, bp=base pairs, nc=negative control (ddw). C-D, CSF smear images of CSF isolated from spontaneous macrometastases-bearing mice, 10 fields per sample were analyzed, n=3. Scale bars=100µm. E-F, Representative TEM images of extracellular vesicles in CSF from normal mice (E), or from injected mice (F). Scale bars=100nm.

**Supplementary Figure 6: *No macrometastases were detected in mice analyzed for blood vessel permeability.*** A, Mice were analyzed by Maestro-CRI imaging for the presence of metastases prior to analyses by Evans blue or FITC-Dextran, 1.5 months after primary tumor removal. Right: mice injected with Evans Blue or FITC-Dextran (n=18). Left: positive control mice bearing experimental macrometastases (n=2). B, Representative mCherry qPCR analysis in the contralateral hemispheres of dye-injected mice or normal mice.

**Supplementary Figure 7: *Brain tropic melanoma cells (BT-RMS) have a more aggressive phenotype.*** A, Schematic summary of the selection of brain-tropic melanoma variants by two rounds of intra-cardiac injections of cells isolated from brain metastases (n=10 in each round, percentage of brain metastases is indicated). B, Validation of brain tropism was performed by subdermal injections. 5·105 RMS or BT-RMS were injected subdermally to 6w old male C57BL/6 mice. Local tumor growth was measured by calipers. Error bars represent SEM (n=15 in each group). n.s, non-significant. C, Spontaneous brain metastasis were detected by FACS analysis of mCherry in brains of injected mice, 2 months after primary tumor removal. Numbers of mice in each group are indicated. D-E, *The PI3K signaling pathway is activated in BT-RMS cells*. D, RMS or BT-RMS cells were incubated in SFM for 48h. pAKT activation was analyzed by western blot. E, Quantification of band intensity shown in D. Results were normalized to total AKT (tAKT) and to RMS. F, *The PI3K signaling pathway is activated in melanoma cells by astrocytes.* Western blot analysis of RMS cells at different time points following incubation with activated-astrocytes CM.G, Quantification of band intensity shown in F. Results were normalized to tAKT and to SFM. D-G are representative of two independent experiments.