Supplementary Figure legends

Figure S1:

1A) Genomic changes in low and high metastatic A549 cells were analyzed by 500K SNP arrays. A large number of genomic alterations were present in A549 cells when compared with the reference genome. On the other hand, no significant differences were observed between the low or high metastatic A549 cell lines.

1B) Methylation levels of single CpG-sites with at least 10 reads coverage in RRBS were compared with Infinium 27K methylation bead array data. The smoothed scatter plot visualizes the methylation levels in RRBS (x-axis) and the respective methylation levels in Infinium methylation bead arrays (y-axis). Colors represent the density of points ranging from red (high density) to blue (low density). For each sample at least 3660 and up to 6519 single CpG-sites were included in the analysis.

1C) The scatter plot depicts differential methylation of differentially methylated regions in A549 and HTB56 which were determined with RRBS in comparison to differential methylation of at least one CpG on the Infinium 27K methylation bead arrays.

Figure S2

2A) Chart depicts viability of high and low metastatic cells before 5-Azacytidine-treatment and after 7 days of 5-Azacytidine-release. HTB56 cells were more sensitive to the toxic effects of 5-Azacitidine.

2B) Analysis of double strand breaks by H2AX-stained parental and higly metastatic cells after six days of Azacytidine (Aza)-treatment and release in normal media for additional seven days. Only a

slight increase of double strand breaks was observed. Note both cell lines showed double strand breaks even without treatment.

2C) Global DNA methylation analysis was performed by liquid chromatography. For A549 cells total DNA methylation was reduced by 5-Azacytidine and slowly increased on day 13.

2D) Proliferation assays were performed in HTB56 cells not exposed or 5-Azacytidine exposed and released cells. The proliferation rate was 2-fold increased in the high metastatic HTB56 cell line compared to the low metastatic control ($p < 0.05$). After 5-Azacytidine exposure and a period of release the highly metastatic cells showed a significant decrease in proliferation.

2E) The highly metastatic cell lines A549 and HTB56 were exposed to a low dose of Azacytidine (100 nM) and released for seven days in media without Aza. Cells were injected into NOD-Scid mice. Metastases formation was observed in all mice to the same extend as in controls. Highly metastatic HTB56 cells treated with a low dose of Azacytidine forms big lung nodules as the controls, whereas low dose treated highly metastatic A549 cells showed small, but many metastases.

Figure S3

Unsupervised hierarchical clustering shows that independent samples at each step of selection cluster more closely together than individual cell lines A549 (A) and HTB56 (B). A high correlation between biological replicates from normal and highly aggressive cell lines from both cell types were observed. The clustering is based on a distance matrix, which has been derived from pearson product-moment correlation coefficients (r) by calculating 1-r.

Figure S4

4A+B): Column charts depict hypo- and hypermethylated regions detected by RRBS in high metastatic A549 (A) and HTB56 (B) cells after 6 days of 5-Azacytidine-treatment and after 7 days of 5-Azacytidine-release.

4C+D) Smoothed scatter plot of 5-Azacytidine-treated (250nM) versus untreated high metastatic HTB56 cells. Shown are methylation levels for CpG-sites analyzed by RRBS.

C: X-axis: Methylation levels in HTB56 high metastatic cells (d0=untreated)

Y-axis: Methylation levels in HTB56 high metastatic cells (d6_250= after six days of 5-Azacytidine-treatment, 5-Azacytidine-concentration of 250 nM)

D: X-axis: Methylation levels in HTB56 high metastatic cells (d0=untreated)

Y-axis: Methylation levels in HTB56 high metastatic cells (d13_250= after thirteen days of 5-Azacytidine-treatment, 5-Azacytidine-concentration of 250 nM)

Figure S5

Unsupervised hierarchical clustering shows that 5-Azacytidine exposed cells cluster more closely together than individual cell lines.

Figure S6

Chromosomal distribution of clusters and DMRs. The curves show the density distribution of hypomethylated DMRs and CpG clusters in relation to their chromosomal position: 0 indicates chromosomal end and 1 the centromeric region. Hypomethylated DMRs (green), hypermethylated DMRs (red) and CpG clusters (black) are shown. DNA demethylation upon 5-Azacytidine occurs primarily on chromosome ends.

A1: Shown is the distribution of the clusters and DMRs for single chromosomes after treating high metastatic A549 cells for 6 days with 250 nM of 5-Azacytidine when compared to untreated cells.

A2: Distribution of clusters and DMRs averaged over all chromosomes after treating high metastatic A549 cells for 6 days with 250 nM of 5-Azacytidine when compared to untreated cells.

B1: Distribution of the clusters and DMRs for all single chromosomes after treating high metastatic A549 cells for 6 days with 1000 nM of 5-Azacytidine when compared to untreated cells.

B2: Distribution of clusters and DMRs averaged over all chromosomes after treating high metastatic A549 cells for 6 days with 1000 nM of 5-Azacytidine when compared to untreated cells.

C1: Distribution of the clusters and DMRs for all single chromosomes after treating high metastatic HTB56 cells for 6 days with 250 nM of 5-Azacytidine when compared to untreated cells.

C2: Distribution of clusters and DMRs averaged over all chromosomes after treating high metastatic HTB56 cells for 6 days with 250 nM of 5-Azacytidine when compared to untreated cells.