

**Supplementary Figure 1 – Multifunctionality deduced from homologous PM\*/SM\* pairs and determined for EC classes; BLAST cutoff 1E-10**

The nodes represent the six EC classes and arrows indicate the relation of functional difference  $PM^* \rightarrow SM^*$ . The width of the arrows represents the number of BLAST hits of enzymes from  $enzymes_{SM^*}$  in  $enzymes_{PM^*}$  and their color the mean E-value; hits were binned as indicated. In addition, for each class, the number of PM BLAST hits is given and the rate of functional conservation  $fc$ , which is the fraction of PM BLAST hits that belong to the same EC class as the SM queries. The class EC 1 subsumes oxidoreductases that catalyze oxidation/reduction reactions and EC 2 transferases that transfer functional groups. EC 3 consists of hydrolases that catalyze the formation of two products from a substrate by hydrolysis and EC 4 contains lyases that catalyze the non-hydrolytic addition or removal of groups. The isomerases of EC 5 catalyze the intramolecular rearrangement within a single molecule and the ligases of EC 6 join together two molecules under consumption of ATP or similar triphosphates. The arrow  $6 \rightarrow 5$  is marked with a  $\blacklozenge$  signals and is the only additional case of neofunctionalization that was not detected by using the cutoff 1E-20 shown in Fig. 2.

