Supplemental Data

Table S1. *In vivo* comparison of RNase activities of four barnase mutants in *E. coli.*

The indicated barnase mutant coding sequence and a non-biologically active mutant coding sequence, barnaseH102Y, were fused with the radiata pine (*Pinus radiata*) *AGAMOUS* promoter, respectively, without barstar in the cloning vector. The resulting constructs were introduced into *E. coli* using the transformation of competent cells. After recovery incubation, series dilutions of the cultures were made and 50 μL from each dilution was placed on a LB agar Petri dish (100x15 mm) supplemented with ampicillin (75 μg mL^{-1}). After overnight (~16 hours) incubation at 37°C, the Petri dishes with similar numbers of single colonies (between 180 and 320) were selected for comparison and the diameters of the single colonies were measured under a dissecting microscope. No single colonies were obtained in the transformation of barnaseK27A construct.

Barnase Mutants	Number of Colonies on a Single Petri Dish (100x150 mm)	Diameter Range of Single Colonies (mm)
BarnasH102Y	245	0.8 – 1.0
BarnasH102E	291	0.8 – 1.0
BarnasE73G	180	0.5 – 0.8
BarnasF106S	283	0.1 – 0.3
BarnasK27A	0	NA

Figure S1. Phenotypes of transgenic tobacco carrying *PrMC2*$_{pro\text{-}I}$*-barnaseH102E*. The photo was taken 60 days after transplanting the tobacco into soil. All tobacco plants in the photo were barnaseH102E tobacco. After 60 days in the glass house, the tobacco plants were about 4- to 5-feet tall with normal-looking leaves, stems and flowers. None of the flowers on all barnaseH102E tobacco plants produced pollen.

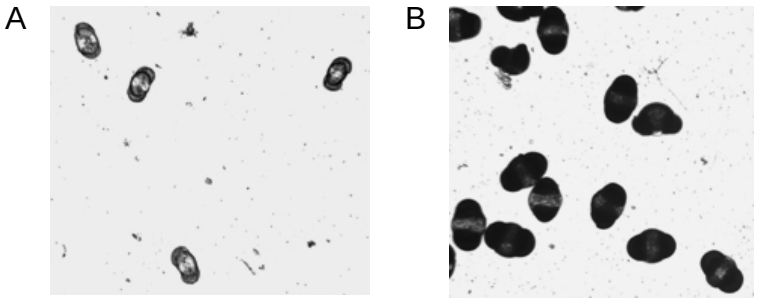

Figure S2. Microscopic observation of normal and under-developed pine pollen grains. A, The pollen grains extracted from an individual male cone of the graft of TRT001343-2 carrying *PrMC2*$_{pro\text{-}I}$*-barnaseH102E*. Most individual male cones harvested from TRT001343-2 did not produce pollen while one or two male cones produces under-developed pollen identified by microscopic observation of dissected individual male cones over three consecutive years. B. The pollen grains extracted from an individual male cone of an untransformed PxL graft at the same grafting site. The magnification is 200-fold for both images.

Figure S3. Phenotypic comparison of barnaseH102E and untransformed pine grafts on potted rootstock trees eight month after grafting.

We grafted 20 transgenic and five untransformed loblolly (*Pinus taeda*) scions on potted and untransformed loblolly trees to evaluate their growth patterns. All 20 transgenic scions contain the pollen ablation cassette of *PrMC2*$_{pro\text{-}II}$*-barnaseH102E* and a second expression cassette for promoting growth. The first two grafts from the left are untransformed grafts while the other two are transgenic grafts carrying a stacking construct pAGK316 (Table I), which is composed of *PrMC2*$_{pro\text{-}II}$*-barnaseH102E* and *Pt4CL-PtUDP-GBD*. At the time of grafting, the scions were about 10 to 15 cm long. Eight month after grafting, the grafts were about 35 to 80 cm long. The blue paint on the potted trees indicates the location of grafting union. The results showed all barnaseH102E grafts are similar to untransformed ones with respect to height, stem diameter, the color of needles and the branching patterns.

Table S2. Number of transgenic lines of tobacco, pine and eucalyptus plants that contained either *PrMC2*$_{pro\text{-}I}$*-barnaseH102E* or *PrMC2*$_{pro\text{-}II}$*-barnaseH102E* cassette and were tested in the glasshouse or in field trials.

Plant Species	**Construct Name**	**Number of transgenic lines tested**	**Number of transgenic lines that produced no pollen**
Tobacco	pWVR220	18	18
Tobacco	pAGF243	12	12
PxL pine	pWVR220	17	16
Loblolly pine	pWVK310	1	1
Loblolly pine	pWVK312	2	2
Loblolly pine	pAGK316	18	18
Loblolly pine	pAGK320	2	2
Loblolly pine	pAGK321	2	2
Eucalyptus occidentalis	pARB598	23	22
Eucalyptus occidentalis	pAGF243	7	7
Eucalyptus EH1	pARB598/pARB599	29	27
Eucalyptus EH1	pABCTE01	12	12